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Supplementary Information for

Ranking essential bacterial processes by speed of mutant death

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Figures S1 and S2
Tables S1 to S4

Other supplementary materials for this manuscript include the following:

Dataset S1

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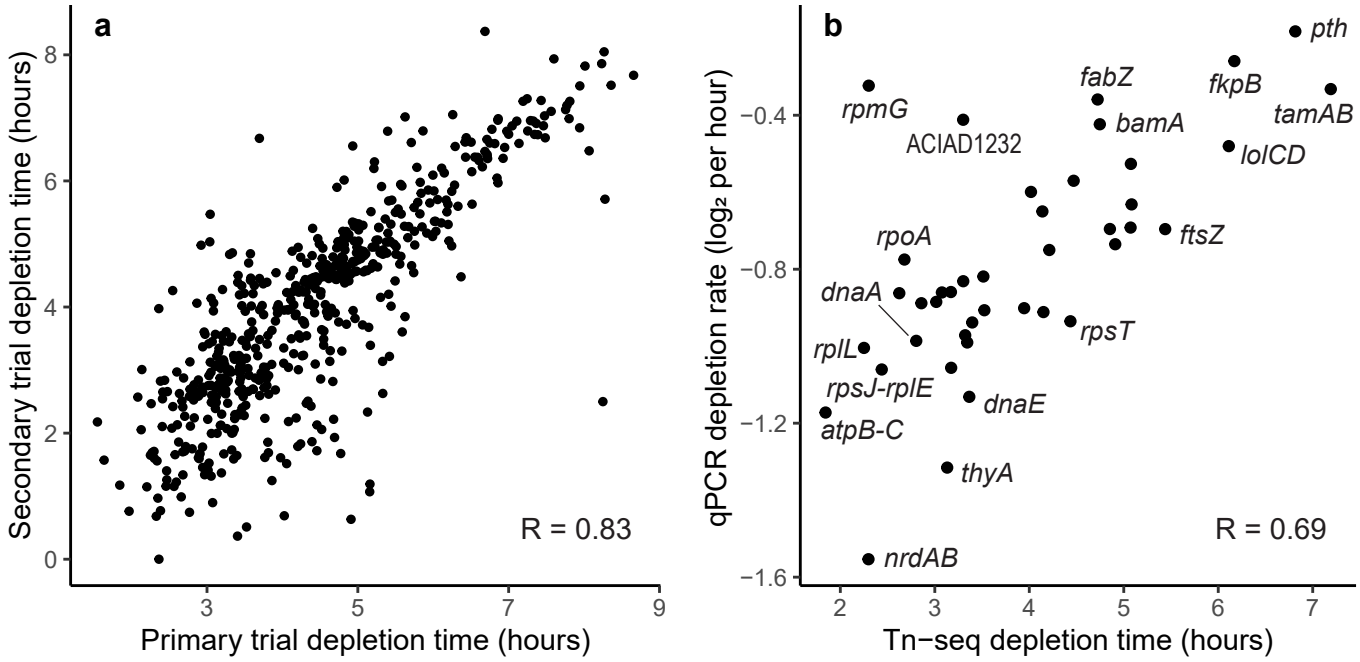

Figure S1. Verification of essential gene mutant depletion times. (**a**) Comparison of the TFNseq two-fold mutant depletion times for the essential genes by two independent transformation and growth trials. R, Pearson's correlation. Most of the prominent outliers correspond to small genes, probably because they were more affected than larger genes by the reduced genome coverage with insertions in the early time points of the secondary trial compared to the primary trial (Table S1). (**b**) Comparison of TFNseq mutant depletion times with depletion rates determined by qPCR for deletions of 40 essential loci (Methods and Table S4). In cases where multiple genes were deleted (e.g., Δ*nrdAB*), the Tn-seq depletion time corresponds to a single representative gene (e.g., *nrdA*) (Table S4). Selected points are labeled with the gene(s) deleted. The TFNseq depletion times were those found in the primary trial.

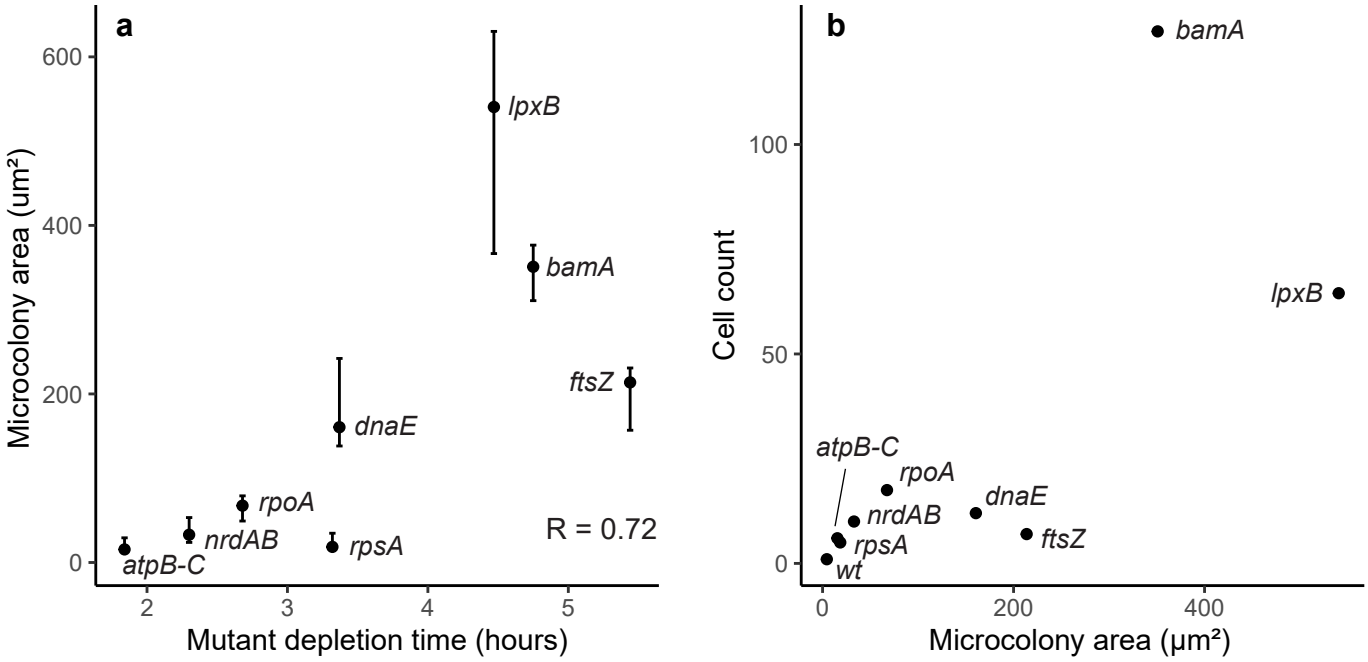

Figure S2. Mutant microcolony size and cell number. (**a**) The median two-dimensional areas of representative deletion mutant microcolonies like those shown in Fig. 5 are plotted against the corresponding TFNseq mutant depletion times. For deletions of multiple genes, the depletion time of the fastest-depleting gene was used. Error bars represent the interquartile range. R, Pearson's correlation. (**b**) The median cell number per microcolony is plotted against the median microcolony area for the microcolonies analyzed in panel a and for a set of wild type microcolonies. The microcolonies of most mutants appeared to consist predominantly of single layers of cells, with the exception of the *bamA* mutant, which appeared mounded. Microcolony areas were measured by manually tracing colony outlines and calculating the corresponding areas using Photoshop (Adobe) (subtracting the areas of large internal spaces devoid of cells such as are seen for *ftsZ* microcolonies). In cases in which mutant cell morphologies were easily distinguished from wild type, outlines did not include the wild type cells. In cases where mutant and wild type cells morphologies were not distinguishable, all cells were included. These areas (for *atpB-C*, *rpsA* and *rpoA*) thus represent maxima for the corresponding mutants. Cells within each microcolony area were counted manually. The numbers of microcolonies analyzed were: 34 wild type, 23 *atpB-C*, 17 *nrdAB*, 20 *rpoA*, 21 *rpsA*, 10 *dnaE*, 16 *lpxB*, 11 *bamA* and 13 *ftsZ*.

Table S1. TFNseq sample summary data.

Sample	Transformants plated at start*	Harvest time (h:min)	Doublings‡	µg gDNA isolated	µg DNA analyzed by Tn-seq	Sequence reads	Number mapped to ADP1	Insertion positions identified	Genes without mutants¶
Pre-transformation sample (mutagenized and repaired genomic DNA)									
m.r.gDNA	NA	NA	NA	NA	1.2	13,172,421	1,332,044†	649,114	(0)
Primary mutant depletion time-course trial									
1°_2h	3.1E+7	2:22	0.5	8.9	8.3	6,957,278	5,152,374	974,353	13
1°_4h	1.5E+7	4:14	3.4	7.4	7.1	3,988,772	2,758,152	329,496	292
1°_6h	4.1E+6	6:08	6.6	6.5	3.5	2,420,406	2,077,341	424,927	523
1°_8h	2.1E+6	8:20	10.0	13.2	2.8	3,263,096	2,924,859	495,539	610
Primary outgrown mutant pool									
AyL4	1.4E+6	18:10	21.4	32.6	1.8	18,824,981	15,600,537	348,758	652
Secondary mutant depletion time-course trial									
2°_2h	1.6E+7	2:16	0.3	4.5	4.5	13,914,130	2,461,365†	126,249	307
2°_4h	7.5E+6	4:10	3.3	3.9	2.8	6,993,141	3,183,592†	181,844	419
2°_6h	3.2E+6	6:06	6.3	3.6	1.6	3,661,566	2,794,729	384,906	526
2°_8h	1.6E+6	8:21	10.3	9.3	1.5	5,337,350	4,264,156	467,628	619
Secondary outgrown mutant pool									
AyL3	7.1E+6	18:00	15.7	24.7	0.4	3,714,372	2,947,731	116,805	689

* Transformants plated: approximate total number of kanamycin-resistant transformants (representing both nonessential and essential genes) plated and grown, based on total kanamycin-resistant cfu in the plating mixture, corrected for the absence of essential gene cfu (565 of 3207 genes are essential, so approximately 82% of transformants would form colonies).

‡ Doublings based on nonessential transposon mutant titer relative to initial titer.

† Many others mapped to T33.

¶ Number of genes depleted at least two-fold relative to the pre-transformation sample.

NA, not applicable.

Table S2. Genes and depletion times for essential processes.

Process	Central essential genes	Other associated genes (inlcuding non-essentials)	Gene depletion times*		
			Median	IQR	Range
ATP synthase	atpA, atpB, atpC, atpD, atpE, atpF, atpG, atpH	adk, atpI, gmk, ndk	**2.3**	2.2 - 2.5	1.8 - 2.6
dNTP synthesis	nrdA, nrdB, thyA	dut, nrdR, tmk	**2.5**	2.4 - 2.8	2.3 - 3.1
Cytochrome oxidase	cyoA, cyoB, cyoC, cyoD, cyoE	-	**2.7**	2.6 - 2.8	2.5 - 4.1
Other energy production	-	cioA, cioB, cydB, etfA, etfB, etfD, etfD, ppa, ybgT	**-**	-	-
Ribosomal proteins	rplA, rplB, rplC, rplD, rplE, rplF, rplJ, rplK, rplL, rplM, rplN, rplO, rplP, rplQ, rplR, rplS, rplT, rplU, rplV, rplW, rplX, rplY, rpmA, rpmB, rpmC, rpmD, rpmE1, rpmF, rpmG, rpmH, rpmI, rpmJ, rpsA, rpsB, rpsC, rpsD, rpsE, rpsF, rpsG, rpsH, rpsI, rpsJ, rpsK, rpsL, rpsM, rpsN, rpsO, rpsP, rpsQ, rpsR, rpsS, rpsT, rpsU	prmA1, prmA2, prmB, rplI, rpmE2	**2.9**	2.4 - 3.4	1.5 - 5.2
NADH dehydrogenase	nuoA, nuoB, nuoCD, nuoE, nuoF, nuoG, nuoH, nuoI, nuoJ, nuoK, nuoL, nuoM, nuoN	-	**3.2**	3 - 3.3	2.9 - 3.5
Pentose Phos. Shunt	rpe, rpiA, tkt	tal	**3.2**	3.1 - 4	3 - 4.8
Histidine synthesis	hisA, hisB, hisC, hisD, hisF, hisG, hisH, hisIE, hisZ	-	**3.2**	3.2 - 3.5	3 - 4.3
Pyrimidine synthesis	cmk, pyrB, pyrC, pyrD, pyrE, pyrF, pyrG, pyrH, pyrX	carA, carB, ndk	**3.2**	3.1 - 4.1	2.3 - 4.2
AA-tRNA Synthetases	alaS, argS, aspS, cysS, gltX, glyQ, glyS, hisS, ileS, leuS, lysS, metG, pheS, pheT, proS, serS, thrS, trpS, tyrS, valS	ACIAD0272, glnS, tilS	**3.3**	3.2 - 3.5	2.9 - 4.4
Fe-S cluster synthesis	ACIAD0010, hscA, hscB, iscA, iscS, iscU	iscR	**3.4**	3.1 - 3.9	2.4 - 4.3
Ile, Leu, Val synthesis	ilvA1, ilvB, ilvC, ilvD, ilvE, ilvN, leuA, leuB, leuC, leuD	-	**3.4**	3.1 - 3.7	2.9 - 4.5
Tryptophan synthesis	aroB, aroC, aroE, aroK, aroQ, trpA, trpB1, trpC, trpD, trpE, trpF, trpG	aroD	**3.4**	3.2 - 4.2	2.8 - 4.9
Purine synthesis	guaA, guaB, purA, purB, purC, purD, purE, purF, purH, purK, purL, purM	adk, gmk, ndk, prs, purN, purT, purU	**3.4**	3.1 - 3.8	2.8 - 3.9
Other AA synthesis	ACIAD2222, asd, glnA, gltB, gltD, glyA, hom, lysA, lysC, pheA, proA, proB, proC, serA, serB, serC, thrC	ACIAD2087, araT, aspC, carA, carB, cysA, cysD, cysE, cysH, cysI, cysK, cysM, cysN, cysP, cysQ, cysT, cysW, lysP, quiA, quiC, sbp, thrH	**3.5**	3.2 - 4.2	3 - 5.5
Redox factors	ACIAD0747, ACIAD1232, ACIAD2244, ACIAD2892, fdx, fdxA, trxA, trxB	ahpC1, ahpC2, grxC, rubA, rubB, trxC	**3.6**	3 - 4.2	2.7 - 6.1
DNA replication	dnaE, dnaN, dnaX, holA, holB, holD, rnhA-dnaQ	ACIAD0352, dnaA, dnaB, dnaG, gyrA, gyrB, holC, hup, ligA, parC, parE, polA, rep, rnhB, ruvA, ruvB, ruvC, ssb, topA	**3.7**	3.5 - 4.4	3.1 - 5.1
TCA Cycle	aceE, aceF, acnB, fumA, gltA, idh, mdh, sdhA, sdhB, sdhC, sdhD, sucA, sucB	ACIAD3189, icd, lpdA1, sucC, sucD	**3.7**	2.7 - 4	2.6 - 5
Gluconeogenesis	ACIAD2287, eno, epd, fba, fbp1, gap, gpmI, pckG, pgk, ppsA, sfcA, tpiA	mqo, pgi, ppc	**3.7**	3 - 5	2.4 - 6.2
Isoprenoid synthesis	dxr, dxs, ispA, ispB, ispD, ispE, ispF, ispG, ispH, uppS	ACIAD1128, ACIAD2405, ubiA, ubiB, ubiC, ubiE, ubiG, ubiH	**4.1**	3.7 - 4.5	3.4 - 6.7
Protein secretion	ffh, ftsY, lepB, oxaA, secA, secD, secE, secF, secY	secB, secG, yajC	**4.2**	3.9 - 4.8	2.4 - 5.4
Arginine synthesis	argA, argB, argC, argD, argF, argG, argH, argJ	argE	**4.2**	3.9 - 4.5	3.2 - 5.8
Translation factors	efp, frr, fusA, infA, infB, infC, prfA, prfB, tsf	ACIAD0208, ACIAD0367, ACIAD0565, ACIAD2561, ACIAD2666, ACIAD3160, engC, era, pcnB, pth, rimM, rnc, rne, rrmJ, smpB, tuf, tuf2	**4.3**	3.4 - 5.5	3 - 6
Methionine synthesis	metE, metX, metZ	ACIAD3222, ACIAD3524, metH, metK, metW, metY	**4.3**	3.9 - 4.7	3.4 - 5.1
Transcription	rpoA, rpoB, rpoC, rpoD	ACIAD2373, greA, greB, mfd, nusA, nusB, nusG, rho, rpoH, rpoN, rpoZ	**4.4**	4 - 4.5	2.7 - 4.5
Riboflavin synthesis	ribA, ribD, ribE, ribF, ribH	ribB1, ribB2	**4.5**	4.2 - 4.8	3.5 - 5.7
tRNA modification	ACIAD1312, ACIAD1895, ACIAD2376, cca, fmt, gatA, gatB, gatC, sirA, trmD, trmU, tusD	sirB	**4.6**	4.2 - 5.4	3.2 - 6.5

Process	Central essential genes	Other associated genes (inlcuding non-essentials)	Gene depletion times*		
			Median	IQR	Range
LPS synthesis	fabZ, kdsA, kdsB, kdsC, kdsD, kdtA, lpxA, lpxB, lpxC, lpxD, lpxH, lpxK, lpxL?	ACIAD0072, ACIAD0073, ACIAD0074, ACIAD0075, ACIAD0081, ACIAD0086, ACIAD1383, glmM, glmS, glmU, lpxL, lpxO	**4.7**	4.2 - 5	3.4 - 6.1
Nicotinamide synthesis	nadA, nadB, nadC, nadE, nadF	ACIAD2606	**4.7**	4.5 - 5	4.4 - 5.9
General regulators	ACIAD0727, ACIAD3019, ACIAD3020, fur, glnK, ihfA, ihfB, ntrC, ompR, rpoH, rpoN, spoT	ACIAD0726, ACIAD0746, ACIAD0985, ACIAD1082, ACIAD1469, ACIAD1519, ACIAD1624, ACIAD1802, ACIAD2627, ACIAD2986, cbl, dctA3, envZ, estR, fis, metR, mlaB, ntrB, relA, typA	**4.8**	4.4 - 5.2	3.6 - 6.8
Chaperones	def1, dnaK, ftsH, groL, groS, grpE	dksA, dnaJ, dsbA, dsbB, fkpB, glnD	**4.8**	4.6 - 5.6	4.2 - 8.1
Fatty acid synthesis	accA, accB, accC, accD, acpP, fabB, fabD, fabG, fabI	ACIAD0585	**4.8**	4.6 - 5.1	4.5 - 5.3
Sodium efflux	phaAB, phaC, phaD, phaE, phaF, phaG	-	**4.9**	4.8 - 4.9	4.7 - 5.1
Heme synthesis	hemA, hemB, hemC, hemD, hemE, hemF, hemH, hemL	cobA	**4.9**	3.9 - 5.5	3.4 - 6
LPS transport	lptA, lptB, lptC, lptD, lptF, lptG	lptE, msbA	**4.9**	4.6 - 5	4.3 - 5.2
Phospholipid synthesis	cdsA, gpsA, pgsA, plsB, psd, pssA	pgpA	**5.0**	4 - 5.4	3.4 - 7.4
PG synthesis	ddlB, mraY, murA, murB, murC, murD, murE, murF, murG, murI2, mviN	dapA, dapB, dapD, dapE, dapF, glmM, glmS, glmU, pbpG	**5.0**	4.7 - 5.1	4.2 - 5.8
Unknown function	ACIAD0041, ACIAD0169, ACIAD0383, ACIAD0508, ACIAD0598, ACIAD0645, ACIAD0659, ACIAD0714, ACIAD0868, ACIAD0878, ACIAD0907, ACIAD0941, ACIAD0944, ACIAD1893, ACIAD1894, ACIAD2010, ACIAD2193, ACIAD2230, ACIAD2926, ACIAD2981, ACIAD3039, ACIAD3280, ACIAD3327, ACIAD3524, ACIAD3568	-	**5.3**	3.8 - 6.9	2.8 - 8.3
Biotin synthesis	(bioH), ACIAD0841, bioA, bioB, bioC, bioD, bioF	-	**5.5**	5.2 - 5.8	3.5 - 6.4
CRISPR	ACIAD2480, ACIAD2481, ACIAD2482, ACIAD2483	ACIAD2477, ACIAD2484	**5.5**	5.2 - 5.8	4.7 - 6.4
Thiamine synthesis	thiC, thiD, thiE, thiG, thiL, thiS	engC, thiM	**5.5**	4.9 - 6	4.7 - 6.8
Folate synthesis	ACIAD2407, folA, folB, folC, folD, folE, folK, folP	metF, pabB, pabC	**5.9**	5.1 - 6.6	3.5 - 7.1
Coenzyme A synthesis	coaBC, coaD, coaE, coaX, panB, panC, panD	-	**6.2**	5.9 - 6.5	5 - 6.7
Pyridoxine synthesis	pdxA, pdxB, pdxH, pdxJ	-	**6.3**	4.9 - 7.6	4.5 - 7.8
Lipoprotein production	lgt, lnt, lolA, lolC, lolD, lspA	lolB	**6.3**	5.2 - 6.8	4.8 - 8.2
OMP localization	bamA, bamD, tamA, tamB	-	**6.9**	6.1 - 7.5	4.7 - 8.3
Cell division	ftsA, ftsI, ftsK, ftsL, ftsN, ftsQ, ftsW, ftsZ	ACIAD0772, ftsB?, ispZ, minC, minD, minE, mrdA, mrdB, mreB, mreC, mreD, rlpA, zapA, zipA	**6.9**	6.3 - 7.4	5.4 - 7.9

*The values shown for depletion times (median, IQR and range) were calculated from the central essential genes listed per process. The depletion time per gene represents the interpolated time of two-fold mutant depletion by Tn-seq relative to its representations in in-vitro-mutagenized genomic DNA during selective growth of bacteria after transformation with*in-vitro* -mutagenized genomic DNA.

Table S3. Essential metabolic reactions predicted to show high flux.

The twelve essential reactions exhibiting the highest flux in the metabolic model for *A. baylyi* ADP1 (1) are shown in decreasing order of flux rate, with the corresponding depletion times and ranks (Dataset S1). For enzymes with multiple subunits encoded by more than one gene, the shortest depletion time is given. Note that the ACIAD2287 product is annotated to be bifunctional.

				Depletion	
Reaction	Process	ACIAD locus	Flux*	Time (h)	Rank (of 565)
ATP synthase	Energy production	0178-0188	999	1.84	3
Fumarate hydratase	TCA cycle	0538	583	4.98	395
Succinate dehydrogenase	TCA cycle	2879-2882	573	2.63	38
Succinate transport	Transport	2227	573	4.08	255
Cytochrome oxidase	Respiration	2425-2428	571	2.53	29
Malate dehydrogenase (NADP)	Other	2287	469	3.64	210
Pyruvate dehydrogenase	Glycolysis	3506, 3507, 2874	426	3.73	220
Glycerol-3-phosphate dehydrogenase	Phospholipid synthesis	1317	300	4.94	387
Phosphate acetyltransferase	Other	2287	283	3.64	210
Malate dehydrogenase (NAD)	TCA cycle	3155	156	4.17	266
Inorganic pyrophosphatase	Other	0239	115	3.70	216
Adenylate kinase	Energy production	1105	102	2.56	32

* Flux (mmoles per gram dry cell weight per hour) was calculated by optimizing the default ADP1 metabolic model (Ref. 1 and https://github.com/opencobra/m_model_collection/blob/master/sbml3/iAbaylyiV4.xml) under the Succinate/Ammonia medium condition using COBRApy (https://opencobra.github.io/cobrapy/).

Reference

1. Durot M, et al. (2008) Iterative reconstruction of a global metabolic model of *Acinetobacter baylyi* ADP1 using high-throughput growth phenotype and gene essentiality data. *BMC Syst Biol* 2:85.

Table S4. Targeted deletions analyzed by qPCR.

Deletion ID	Gene(s) targeted	(Loci)	Del coordinates (bp)* From	To	qPCR depletion rate (log2 / hr)*	Representative gene	Depletion time (h)‡
d101	*rplL*	ACIAD0306	301,788	302,143	-1.00	*rplL*	2.25
d102	*rpmG*	ACIAD0501	494,202	494,087	-0.32	*rpmG*	2.30
d103	*rpsT*	ACIAD1389	1,384,927	1,384,705	-0.94	*rpsT*	4.44
d104	*aspS*	ACIAD0609	599,060	597,301	-0.99	*aspS*	3.34
d105	*glnS*	ACIAD1920	1,912,092	1,910,448	-0.94	*glnS*	3.40
d106	*rpsJ-rplE*	ACIAD3220-07	3,135,082	3,128,718	-1.06	*rpsC*	2.44
d107	*pth*	ACIAD2909	2,845,827	2,846,385	-0.18	*pth*	6.82
d109	*dnaA*	ACIAD0001	206	1,592	-0.99	*dnaA*	2.80
d110	*dnaE*	ACIAD2089	2,072,314	2,075,862	-1.13	*dnaE*	3.37
d111	*nrdAB*	ACIAD0724-2	710,274	705,859	-1.55	*nrdA*	2.30
d112	*thyA*	ACIAD0515	503,742	502,943	-1.32	*thyA*	3.13
d113	*pyrBX*	ACIAD1270-1	1,270,637	1,272,840	-0.89	*pyrB*	2.86
d114	*pyrC*	ACIAD1150	1,141,940	1,140,914	-0.65	*pyrC*	4.14
d115	*gltA*	ACIAD2886	2,825,327	2,826,561	-0.60	*gltA*	4.02
d116	*atpB-C*	ACIAD0180-8	179,051	185,716	-1.17	*atpB*	1.84
d118	*cyoA-E*	ACIAD2425-9	2,391,462	2,396,317	-0.86	*cyoB*	2.62
d119	*fabI*	ACIAD3116	3,047,119	3,046,356	-0.53	*fabI*	5.08
d120	*fabZ*	ACIAD1381	1,377,591	1,378,064	-0.36	*fabZ*	4.72
d121	*plsB*	ACIAD3232	3,146,192	3,148,716	-0.63	*plsB*	5.08
d122	*mraY*	ACIAD3363	3,272,225	3,271,112	-0.69	*mraY*	5.07
d123	*lpxB*	ACIAD2324	2,287,784	2,286,633	-0.57	*lpxB*	4.47
d124	*lolCD*	ACIAD2641-0	2,596,525	2,594,708	-0.48	*lolD*	6.11
d125	*bamA*	ACIAD1378	1,373,490	1,375,898	-0.42	*bamA*	4.75
d126	*tamAB*	ACIAD2403-2	2,365,643	2,358,438	-0.33	*tamB*	7.19
d127	*ftsZ*	ACIAD3511	3,440,129	3,438,965	-0.70	*ftsZ*	5.44
d128	*secA*	ACIAD0648	636,816	639,526	-0.91	*secA*	4.15
d129	*ffh*	ACIAD0839	826,407	827,805	-0.90	*ffh*	3.95
d130	*ftsY*	ACIAD2296	2,268,235	2,267,155	-0.83	*ftsY*	3.30
d131	*iscS-hscA*	ACIAD1404-1399	1,399,211	1,394,724	-1.06	*hscA*	3.17
d132	*fkpB*	ACIAD0020	26,234	25,761	-0.26	*fkpB*	6.17
d133	-	ACIAD1232	1,233,154	1,233,456	-0.41	ACIAD1232	3.30
d134	*groSL*	ACIAD2839-8	2,777,281	2,775,325	-0.70	*groL*	4.85
d135	*ftsH*	ACIAD2853	2,791,719	2,789,836	-0.75	*ftsH*	4.21
d136	-	ACIAD0878	861,463	861,017	-0.74	ACIAD0878	4.91
d137	*etfBA*	ACIAD2655-4	2,611,316	2,609,666	-0.86	*etfA*	3.17
d138	-	ACIAD3524	3,452,832	3,451,902	-0.82	ACIAD3524	3.51
d139	-	ACIAD2244	2,214,833	2,215,580	-0.88	ACIAD2244	3.02
d140	-	ACIAD2981	2,910,152	2,909,679	-0.86	ACIAD2981	3.08
d142	-	ACIAD2153-1	2,136,777	2,132,849	-0.26	ACIAD2153	(non ess)
d143	-	ACIAD2802-2799	2,748,143	2,745,775	0 (reference)	ACIAD2799	(non ess)
d145	*rplS*	ACIAD3310	3,217,401	3,217,058	-0.91	*rplS*	3.52
d147	*rpoA*	ACIAD3194	3,122,923	3,121,922	-0.77	*rpoA*	2.68
d148	*rpsA*	ACIAD2347	2,310,668	2,309,024	-0.97	*rpsA*	3.32

* The sequences of the primers used for amplification of the target flanking sequences and for deletion detection by qPCR are available upon request.

‡ TFNseq depletion times were those found in the primary trial.