Supplementary Material for:

Combinatorial strategies for improving multiple stress resistance in industrially-relevant *Escherichia coli* strains

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Supplementary Methods: Error analysis for background strain stress characterization

Growth rates, lag times, and final densities were calculated as described in Materials and Methods using growth curves averaged over all biological replicates. To determine standard errors ($\delta\mu$) for growth rates (μ), standard deviations (δX_i) about each mean measurement (X_i) used in the determination of the growth rate were propagated according to:

$$\delta\mu = \sqrt{\sum_{i=1}^{n} \left(\frac{\partial\mu}{\partial X_i}\right)^2 (\delta X_i)^2}$$

For a linear equation with equally spaced measurements in X:

$$\left(\frac{\partial\mu}{\partial X_1}\right) = \left(\frac{\partial\mu}{\partial X_2}\right) = \cdots = \left(\frac{\partial\mu}{\partial X_n}\right)$$

Thus we can write:

$$\mu = \ln(X - X_0)\,/(t - t_0)$$

$$\frac{\partial\mu}{\partial X} = \frac{1}{\Delta X \Delta t}$$

Therefore the standard errors in growth rate were determined as:

$$\delta\mu = \frac{1}{\Delta X \Delta t}\sqrt{\sum_{i=1}^{n} (\delta X_i)^2}$$

Final densities (X_{final}) are reported as the average of averages over n timepoints, therefore the standard errors (δX_{final}) can be similarly propagated as:

$$\delta X_{final} = \frac{1}{n}\sqrt{\sum_{i=1}^{n} (\delta X_i)^2}$$

A MATLAB script was written to perform these calculations using the actual time intervals selected for determination of growth rates and final densities for each averaged growth curve. For relative growth rates and densities, an additional propagation for arithmetic operations to calculate percentages was performed.

Table S1. Oligonucleotide primers and adapters used in this study.

	Primer name	Sequence (5' to 3')[a,b]
1	Tn5_InvPCR_f1	GCAGAGCATTACGCTGACTTGAC
2	Tn5_InvPCR_f2	GCTCGATGAGTTTTTCTAATCAGAATTGG
3	Tn5_InvPCR_f3	TATCCTGATATGAATAAATTGCAGTTTCATTTG
4	Tn5_InvPCR_r1	CTATCAACAGGTTGAACTGCTGATC
5	Tn5_InvPCR_r2	TCGGATCTATGTCGGGTGCG
6	Tn5_InvPCR_r3	GTACGTGAAACATGAGAGCTTAGTAC
7	prc-Tn5_fwd	TATCTTCGAATTTCTCACCCGTTTC
8	prc-Tn5_rev	GTGTTCTATAAAGGCCCGCTG
9	rfe-Tn5_fwd	TGTGCCGGTGTGCTTGTTTTC
10	rfe-Tn5_rev	CAACTTTCCAGGCACGCTTAATG
11	slp-Tn5_fwd	CTATAACCTGTGGGATTACGGC
12	slp-Tn5_rev	AACAAAGGTCACCGGCACTTTC
13	typA-Tn5_fwd	CTGTAATGCAGGCGCTGGG
14	typA-Tn5_rev	CACCGTGACCGAGGAACAG
15	nanM-Tn5_fwd	CTATTGGTTTTGGGGTTGTATTTGTG
16	nanM-Tn5_rev	ATGAATAAAACAATAACGGCGCTTGC
17	gltB-Tn5_fwd	AACCCGGAACTGGTCGAGG
18	gltB-Tn5_rev	AAAACGGCTCGTAAATTTCAACAAACTC
19	acrB-Tn5_fwd	CGAACCGTACTCCCAACGAG
20	acrB-Tn5_rev	GTAACGCATTACTATCTGACCAAAG
21	yeeO-Tn5_fwd	CAACCACACCCCAGCCAAG
22	yeeO-Tn5_rev	TTATTAACCCAAATGTTCGTTGCCG
23	nagA-Tn5_fwd	ACCGTTAACGATGGTCTTGGTG
24	nagA-Tn5_rev	GATTACGCCAACATTCGTAACGC
25	dgoD-Tn5_fwd	AGGTTAGCGCGGTCAACATAAC
26	dgoD-Tn5_rev	CGACTTTGTTTCCTATAACGCGG
27	rnk-Tn5_fwd	GAGCCATAACGGAAAGCTGAG
28	rnk-Tn5_rev	GTGGGTTATTAATCATCTGATTTACAAAAC
29	ptsP-Tn5_fwd	CAACTGCGACTGAGAAAGAATAGC
30	ptsP_Tn5_rev	AAAGGTAGCCAGCGCACCAC
31	proV-Tn5_fwd	TTGAAGAAGGCGAGATATTTGTCATC
32	proV-Tn5_rev	GCCTGTAATTTTACCAGCTCATCC
33	NevgS-Tn5_fwd	AAGTTTTTACCCTATTTTTTTCTTCTCTGTTG
34	NevgS-Tn5_rev	GAAATCATACTGCTGGTAATGATGTTAC
35	CevgS-Tn5_fwd	CCAGCAAGTGGCGACTGTC
36	CevgS-Tn5_rev	GTGGCGATACTGAGGTGCAATAT
37	evgA-Tn5_fwd	TAGGATTAGTAAGAAGACTTATAGTGCC

1	38	evgA-Tn5_rev	AATGATGACGATATCAGGCTTAAGTG
2	39	gltI-Tn5_fwd	GTTCAGTGCTTTGTCATTCGGTTC
3	40	gltI-Tn5_rev	CCAAAGATCACGGTGACTCTTTC
4	41	ytfL-Tn5_fwd	GGAACTCGTCAATATCCAGCAC
5	42	ytfL-Tn5_rev	GGTTTGATCTCCACGAAGATGAG
6	43	ygjI-Tn5_fwd	CTCAGAAGCCGGTGTCTACG
7	44	ygjI-Tn5_rev	GCCATAAAGATCCAGGTGGTAAC
8	45	nagC-Tn5_fwd	CTGGAGCAAAATACCGTTGAGC
9	46	nagC-Tn5_rev	TTGGCCATATTCAGGTCGAACC
10	47	yobF-Tn5_fwd	GATAGCGGAGAAGTGTACGAAC
11	48	yobF-Tn5_rev	GTGGAAGACGTATCGAGATTTGTG
12	49	ackA-Tn5_fwd	CCTGGGCAACGGTGGTTC
13	50	ackA-Tn5_rev	CATCAGCGCAGTGTAGGCAC
14	51	rcsB-Tn5_fwd	GAAGGGATCGTGCTGAAACAAG
15	52	rcsB-Tn5_rev	GAAGAGAGATAATTCAGCAGGGC
16	53	PyliE-Tn5_fwd	GGTTATCGCGCCGCATGTC
17	54	PyliE-Tn5_rev	ATAACTATCTGAACGCTGTGCCG
18	55	P1m0088-Tn5_fwd	CAGCTACGGGATCTTGTCGG
19	56	P1m0088-Tn5_rev	ATACCGGAACCTGCTTACCAATC
20	57	mutS-Tn5_fwd	AAACCGCCAATATTTTACATAACGCC
21	58	mutS_Tn5_rev	GACATTTGCGTACCATCCACTTG
22	59	mutL-Tn5_fwd	CTGGCAGAAATTCAGTATTGCTAC
23	60	mutL-Tn5_rev	GCCGGTTCTGCAAAGTGATTG
24	61	ygaH-Tn5_fwd	GCTCTGCTGGTTGTCTCTAC
25	62	ygaH-Tn5_rev	GGCGCGAAATTTTAGCATTTGTTC
26	63	yciW-Tn5_fwd	TTAATATGTCTCACCCAACGCGATT
27	64	yciW-Tn5_rev	CACTCTCTCTCCACCCTATTTC
28	65	proV_KO_fwd	GTAATATATCGACATAGACAAATAAAGGAATC
29	66	proV_KO_rev	CGCTGGCGTGGTATCCC
30	67	proV_colPCR_fwd	GATTTGCTCGCATCAATATTCATGC
31	68	proV_colPCR_rev	CACCACCGCCGTCAGTC
32	69	rfe_KO_fwd	GGTCTTCGTGGTTATACTTCTGC
33	70	rfe_KO_rev	ATTGGTTGTGTCATCACATCCTCAT
34	71	rfe_colPCR_fwd	TTGCATATCAAATGGTTAATTTTTGCACAG
35	72	rfe_colPCR_rev	CGCCAGCCCCATGCCAAT
36	73	slp_KO_fwd	CTATTATGGTTTTAATATTTGTTGATAAGGATAG
37	74	slp_KO_rev	CATCTGCATCTTTCGGTGGTG
38	75	slp_colPCR_fwd	GGATATAAACATCAGACAGGTTTACG
39	76	slp_colPCR_rev	CTGGTGCGGACATTAACTTTATAATC
40	77	dctR_KO_fwd	GTTAATTACCTTTGGCAAACTGATTATAAAG
41	78	dctR_KO_rev	CAGACTCACCGTAAGCCTGAAAT

1	79	dctR_colPCR_fwd	GTAAGCTAACTATTATTATTATAAGCCCTG
2	80	dctR_colPCR_rev	AAGTGGTCGCCATTGATATCCAG
3	81	evgS_KO_fwd	GCCTGATGGAAAAATTAGAATGTAAATCAC
4	82	evgS_KO_rev	TTTGAACATTGTGGGAGCCGCTAT
5	83	evgS_colPCR_fwd	ATGGCAAGGATAATAATGACATTGC
6	84	evgS_colPCR_rev	GAACAAATTCGCCAGGAGTTCTC
7	85	evgA_KO_fwd	TTACTACAGGGAGAAGGGAGATG
8	86	evgA_KO_rev	GGGTAAAAACTTCATGTGATTAGCCGATTTTGTTACGTTGTGTAG
9	87	evgA_colPCR_fwd	CCGACTATTTATATGGTATACTTGTCG
10	88	evgA_colPCR_rev	CGTAATTCCTTGTTGCTCAGACG
11	89	typA_KO_fwd	CCGTGTACAATAACGCGCTATTTC
12	90	typA_KO_rev	CCATCGCTGGCAGGTTTTTTATG
13	91	typA_colPCR_fwd	TGACCTTTGGATAACCCTTTTTATGC
14	92	typA_colPCR_rev	GACAAAGCTCTCTATTGACGTAACC
15	93	gltI_KO_fwd	CGGGTATCCATGCGTTCTTAAC
16	94	gltI_KO_rev	TGTCCGTGCTACGTAACAATCG
17	95	gltI_colPCR_fwd	CTAACAGGCACAACACTGCAC
18	96	gltI_colPCR_rev	AGATTGTTACCCAGCGTATTGCG
19	97	nanM_KO_fwd	GTTATCGCATTGGTGTGTCATTTAAAC
20	98	nanM_KO_rev	CGCGTAATTGAAATGACAAATTGATAGC
21	99	nanM_colPCR_fwd	AATACCATATGACGCCCGATATTAC
22	100	nanM_colPCR_rev	CCAGCAACGGTAAGAACATAGTAATA
23	101	gltB_KO_fwd	TAACCGATGCGAAAAGGACAACAAGGGGGCGAATGCGAGGCGCGCGTAT
24			GATTCCGGGGATCCGTCGACC
25	102	gltB_KO_rev	TAAACATTCTGACTCATTGTTGCTACCCCTTACTGCGCCTGCACGCGCA
26			ATGTAGGCTGGAGCTGCTTCG
27	103	gltB_colPCR_fwd	CACCGTATTAACCGATGCGAAAAG
28	104	gltB_colPCR_rev	CGGCTCGTAAATTTCAACAAACTCAAT
29	105	ytfL_KO_fwd	GTATTTCACACAAATTAATCAACTTCCCC
30	106	ytfL_KO_rev	TATGCAGTAATAAGACGGCTCCTG
31	107	ytfL_colPCR_fwd	CTTTACAGTACCTTACGCTATACTAG
32	108	ytfL_colPCR_rev	CATTTGTCAGTGATGTCCGAAGTTAA
33	109	nagC_KO_fwd	ATTTTAAAATCACCAAGACCATCGTTAAC
34	110	nagC_KO_rev	ATGGACTACCCAGAATATTGACAAC
35	111	nagC_colPCR_fwd	TTGAGAAACGTCTCGGCACAC
36	112	nagC_colPCR_rev	AATCCCGTGCAAAAATTCCGCTG
37	113	nagA_KO_fwd	TGGCTCCTTGCTCAGGGCAATATTTTTAAAATCGGGGGTCAGAATGATT
38			CCGGGGATCCGTCGACC
39	114	nagA_KO_rev	AGCTTGTCCGCCTGGTGTCATACTTTCTCTTATTGAGTTACGACCTCGT
40			TTGTAGGCTGGAGCTGCTTCG
41	115	nagA_colPCR_fwd	TGCGATGAACCTTCCACCATG

1	116	nagA_colPCR_rev	GCTTTGCTCGGCAATCTGAATC
2	117	yobF_KO_fwd	GCGCGTATTCCGTTGCATAAG
3	118	yobF_KO_rev	TTGAACCACTTAACCTGACCTTTAATC
4	119	yobF_colPCR_fwd	CTGATCGAGACATGTTTAAAAATGGC
5	120	yobF_colPCR_rev	TTCAGCCAGAGTTTTGAAGCCATTA
6	121	ygjI_KO_fwd	AGTTTGCCGCCACCGCTAC
7	122	ygjI_KO_rev	CAGGAAGAGGGAAAATGCCTG
8	123	ygjI_colPCR_fwd	AAGCGTATCGGTTTATCTGCAATAAC
9	124	ygjI_colPCR_rev	GCAGTGATTAATTTCATTGTGTTAATCC
10	125	acrB_KO_fwd	GCCTGAACAGTCCAAGTCTTAAC
11	126	acrB_KO_rev	TGCATAAAAAAGGCCGCTTACGC
12	127	acrB_colPCR_fwd	GCGTAGTAATAAGTGGGCTGC
13	128	acrB_colPCR_rev	GGTTAATACTGGTTTTCGTATGAGATC
14	129	yeeO_KO_fwd	ACGGATTAACAGTCCGCCGTTCTACCGACTGAACTACAGAGGAATCGTT
15			GATTCCGGGGATCCGTCGACC
16	130	yeeO_KO_rev	GTTTGCTGAAATAATCTGCATTTTGTCGTTTATTCCGACACAACTGGCT
17			TTGTAGGCTGGAGCTGCTTCG
18	131	yeeO_colPCR_fwd	GCAGTGAAGAGGAAATTGATTATCAG
19	132	yeeO_colPCR_rev	AGTCCGCCGTTCTACCGAC
20	133	dgoD_KO_fwd	CAGGCAGCAGCATTTGTTAAGG
21	134	dgoD_KO_rev	TGCTGTTTTAGTGCCCGATGAG
22	135	dgoD_colPCR_fwd	AAAACCTGGCGCAGTGGATAG
23	136	dgoD_colPCR_rev	CCGGCTTTGCTGCATTAACGG
24	137	rnk_KO_fwd	TCTGCGACACTCGCAGTACCGACGACATGGAGTAAAAATG
25	138	rnk_KO_rev	ATCCTCTGGGCAAAGCGAGTT
26	139	rnk_colPCR_fwd	TCCCTGAATGTGACGCAAATCAC
27	140	rnk_colPCR_rev	TGCCATGGTTATTCCCACAAACG
28	141	ptsP_KO_fwd	CCACAAAACGCATCTGCTTATCG
29	142	ptsP_KO_rev	CGAATAATAGCACAAGGGGACC
30	143	ptsP_colPCR_fwd	GTGGTGTCATTTAAACGTGATGTC
31	144	ptsP_colPCR_rev	CTGTCACCACAAGTTCTTGTTATTTC
32	145	ackA_KO_fwd	TGGCTCCCTGACGTTTTTTTAGCCACGTATCAATTATAGGTACTTCCAT
33			GATTCCGGGGATCCGTCGACC
34	146	ackA_KO_rev	AGCTGAGCTGGCGGTGTGAAATCAGGCAGTCAGGCGGCTCGCTGTAGGC
35			TGGAGCTGCTTCG
36	147	ackA_colPCR_fwd	CAAAATGGCATAGACTCAAGATATTTC
37	148	ackA_colPCR_rev	CGGTAGGGATCAGCATAATAATAC
38	149	rcsB_KO_fwd	ACAGTTATGTCAAGAGCTTGCTGTA
39	150	rcsB_KO_rev	TGCCAGATAAGACACTAACGCGTCTTATCTGGCCTACAGATGATTAGTC
40			TTTATCTGCCGGACT
41	151	rcsB_colPCR_fwd	AAATCTGGTACCCGGCAAGC

152	rcsB_colPCR_rev	ACGCGTCTTATCTGGCCTAC
153	P1m0088_KO_fwd	GAGTGTATTGTTATTATTAAATACGCAGTGTGACAAGTGAGCAAAAGAT GATTCCGGGGATCCGTCGACC
154	P1m0088_KO_rev	TTTCTCTCTGATTAAACGAGCCGACCGGTTCAGAAATCAAATTCTATTG CTGTAGGCTGGAGCTGCTTCG
155	P1m0088_colPCR_fwd	GTCTAAACTGCAGGCTTTGTCG
156	P1m0088_colPCR_rev	GTGTCATCGCTGCGAAAGTTG
157	yciW_KO_fwd	CCGCCTTGCGTCAGGATAACGATTTCCTTTACGACCAAGGAGCGCCCAT GATTCCGGGGATCCGTCGACC
158	yciW_KO_rev	TTTACGCGAATCTGTCTGACGCGGCAAGGTTAATATGTCTCACCCAACG CTGTAGGCTGGAGCTGCTTCG
159	yciW_colPCR_fwd	CGTTCTGCCGCCCGTTATC
160	yciW_colPCR_rev	TTACCACCCCTTCAGCGCG
161	ygaH_KO_fwd	CACTGCGTTAATCCAGGCATTCTGGCAAGGAGCGCCCGATGAGCTATGA GATTCCGGGGATCCGTCGACC
162	ygaH_KO_rev	TATTGTTTTAGAAAAATGATTCTTGTGGGTTATATAATCGCCATCACTT TTGTAGGCTGGAGCTGCTTCG
163	ygaH_colPCR_fwd	TCCAGCGCAAACAATCTCTTTGC
164	ygaH_colPCR_rev	CGGCTGGCGCGAAATTTTAG
165	Keio_kan_fwd	ATACGCTTGATCCGGCTACCT
166	BioTEG-Tn5seq_fwd[c,f]	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC TTCCGATCTCAGGCATGCAAGCTTCAGGGTTG
167	Tn5seq_rev[g]	CAAGCAGAAGACGGCATACGAGAT
168	UAD_tail[d,f]	GCTCTTCCGATCT
169	AD002[e,g]	GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATG CCGTCTTCTGCTTG
170	AD006[e,g]	GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATG CCGTCTTCTGCTTG
171	AD012[e,g]	GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAATCTCGTATG CCGTCTTCTGCTTG
172	AD015[e,g]	GATCGGAAGAGCACACGTCTGAACTCCAGTCACATGTCAGAATCTCGTA TGCCGTCTTCTGCTTG
173	AD005[e,g]	GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATG CCGTCTTCTGCTTG
174	AD007[e,g]	GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGATCATCTCGTATG CCGTCTTCTGCTTG

[a] Primers containing 'KO' in the name were used for amplification of cassettes derived from pKD13 from Keio collection strains. Primers containing 'colPCR' were used colony PCR verification of chromosomal gene insertions and deletions.
[b] 5' overhangs during PCR amplification are underlined
[c] Contains a 5'-biotin-tetraethyleneglycol (TEG) modification
[d] Contains a 3'-phosphorothioate modification between the final two nucleotides
[e] Contains a 5'-phosphate modification

[f] Oligonucleotide sequences © 2007-2012 Illumina, Inc. All rights reserved. Derivative works created by Illumina customers are authorized for use with Illumina instruments and products only. All other uses are strictly prohibited.

[g] Oligonucleotide sequences © 2007-2012 Illumina, Inc. All rights reserved.

Table S2. Additional strains used in this study.

Strain	Relevant genotype/property[a]	Source/ Reference
JW2652-1	BW25113 *proV::kan*	1
JW3758-1	BW25113 *rfe::kan*	1
JW3474-1	BW25113 *slp::kan*	1
JW3475-1	BW25113 *dctR::kan*	1
JW5571-1	BW25113 *typA::kan*	1
JW2367-1	BW25113 *evgS::kan*	1
JW2366-1	BW25113 *evgA::kan*	1
JW2205-1	BW25113 *rcsB::kan*	1
JW5092-1	BW25113 *gltI::kan*	1
JW5777-1	BW25113 *nanM::kan*	1
JW4177-1	BW25113 *ytfL::kan*	1
JW5512-1	BW25113 *ygjI::kan*	1
JW0451-1	BW25113 *acrB::kan*	1
JW0662-1	BW25113 *nagC::kan*	1
JW1813-1	BW25113 *yobF::kan*	1
JW5629-1	BW25113 *dgoD::kan*	1
JW0602-1	BW25113 *rnk::kan*	1
JW2797-1	BW25113 *ptsP::kan*	1
RL136	BL21(DE3) *proV*::*kan*	This work
RL137	W *rfe*::*kan*	This work
RL138	W *slp*::*kan*	This work
RL139	W *dctR*::*kan*	This work
RL140	W *typA*::*kan*	This work
RL141	W *evgS*::*kan*	This work
RL142	W *evgA*::*kan*	This work
RL143	W *rcsB*::*kan*	This work
RL144	BL21(DE3) *gltI*::*kan*	This work
RL145	BL21(DE3) *nanM::kan*	This work
RL146	BL21(DE3) *gltB*::*kan*	This work
RL147	BL21(DE3) *ytfL::kan*	This work
RL148	BL21(DE3) *ygjI::kan*	This work
RL149	BL21(DE3) *acrB::kan*	This work
RL150	BL21(DE3) *yeeO::kan*	This work
RL151	W *nagC::kan*	This work
RL152	W *nagA::kan*	This work

RL153	W *yobF::kan*	This work
RL154	W *dgoD::kan*	This work
RL155	W *rnk::kan*	This work
RL156	W pRK1[ECW_P1m0088::*kan*]	This work
RL157	W *ackA::kan*	This work
RL158	W *ptsP::kan*	This work
RL159	W *yciW::kan*	This work
RL160	W *ygaH::kan*	This work
RL161	BL21(DE3) Δ*proV*	This work
RL162	W Δ*rfe*	This work
RL163	W Δ*slp*	This work
RL164	W Δ*dctR*	This work
RL165	W Δ*typA*	This work
RL166	W Δ*evgS*	This work
RL167	W Δ*evgA*	This work
RL168	W Δ*rcsB*	This work
RL169	BL21(DE3) Δ*gltI*	This work
RL170	BL21(DE3) Δ*gltB*	This work
RL171	BL21(DE3) Δ*ytfL*	This work
RL172	BL21(DE3) Δ*ygjI*	This work
RL173	BL21(DE3) Δ*acrB*	This work
RL174	BL21(DE3) Δ*yeeO*	This work
RL175	W Δ*nagC*	This work
RL176	W Δ*nagA*	This work
RL177	W Δ*yobF*	This work
RL178	W Δ*dgoD*	This work
RL179	W Δ*rnk*	This work
RL180	W pRK1[ΔECW_P1m0088]	This work
RL181	W Δ*ackA*	This work
RL182	W Δ*ptsP*	This work
RL183	W Δ*yciW*	This work
RL184	W Δ*ygaH*	This work
RL185	W Δ*rfe evgA::kan*	This work
RL186	W Δ*rfe typA::kan*	This work
RL187	W Δ*rfe rcsB::kan*	This work
RL188	W Δ*rfe nagC::kan*	This work
RL189	W Δ*rfe ptsP::kan*	This work
RL190	W Δ*rfe yobF::kan*	This work
RL191	W Δ*rfe nagA::kan*	This work

RL192	W Δ*rfe ackA::kan*	This work
RL193	W Δ*rfe ygaH::kan*	This work
RL194	W Δ*rfe yciW::kan*	This work
RL195	W Δ*evgA typA::kan*	This work
RL196	W Δ*evgA rcsB::kan*	This work
RL197	W Δ*evgA nagC::kan*	This work
RL198	W Δ*evgA ptsP::kan*	This work
RL199	W Δ*evgA yobF::kan*	This work
RL200	W Δ*evgA nagA::kan*	This work
RL201	W Δ*evgA ackA::kan*	This work
RL202	W Δ*evgA ygaH::kan*	This work
RL203	W Δ*evgA yciW::kan*	This work
RL204	W Δ*typA rcsB::kan*	This work
RL205	W Δ*typA nagC::kan*	This work
RL206	W Δ*typA yobF::kan*	This work
RL207	W Δ*typA nagA::kan*	This work
RL208	W Δ*typA ackA::kan*	This work
RL209	W Δ*typA ygaH::kan*	This work
RL210	W Δ*typA yciW::kan*	This work
RL211	W Δ*rcsB nagC::kan*	This work
RL212	W Δ*rcsB ptsP::kan*	This work
RL213	W Δ*rcsB yobF::kan*	This work
RL214	W Δ*rcsB nagA::kan*	This work
RL215	W Δ*rcsB ackA::kan*	This work
RL216	W Δ*rcsB ygaH::kan*	This work
RL217	W Δ*rcsB yciW::kan*	This work
RL218	W Δ*nagC ptsP::kan*	This work
RL219	W Δ*nagC yobF::kan*	This work
RL220	W Δ*nagC ackA::kan*	This work
RL221	W Δ*nagC ygaH::kan*	This work
RL222	W Δ*nagC yciW::kan*	This work
RL223	W Δ*ptsP typA::kan*	This work
RL224	W Δ*ptsP yobF::kan*	This work
RL225	W Δ*ptsP nagA::kan*	This work
RL226	W Δ*ptsP ackA::kan*	This work
RL227	W Δ*ptsP ygaH::kan*	This work
RL228	W Δ*ptsP yciW::kan*	This work
RL229	W Δ*yobF nagA::kan*	This work
RL230	W Δ*yobF ackA::kan*	This work

RL231	W Δ*yobF ygaH::kan*	This work
RL232	W Δ*yobF yciW::kan*	This work
RL233	W Δ*nagA ackA::kan*	This work
RL234	W Δ*nagA ygaH::kan*	This work
RL235	W Δ*nagA yciW::kan*	This work
RL236	W Δ*ackA ygaH::kan*	This work
RL237	W Δ*ackA yciW::kan*	This work
RL238	W Δ*ygaH yciW::kan*	This work
RL239	W Δ*rfe* Δ*evgA*	This work
RL240	W Δ*rfe* Δ*typA*	This work
RL241	W Δ*rfe* Δ*ptsP*	This work
RL242	W Δ*evgA* Δ*typA*	This work
RL243	W Δ*evgA* Δ*ptsP*	This work
RL244	W Δ*typA* Δ*ptsP*	This work
RL245	W Δ*evgA* Δ*yciW*	This work
RL246	W Δ*typA* Δ*yobF*	This work
RL247	W Δ*typA* Δ*yciW*	This work
RL248	W Δ*ptsP* Δ*yobF*	This work
RL249	W Δ*ptsP* Δ*yciW*	This work
RL250	W Δ*rfe* Δ*evgA typA::kan*	This work
RL251	W Δ*rfe* Δ*evgA ptsP::kan*	This work
RL252	W Δ*rfe* Δ*evgA yobF::kan*	This work
RL253	W Δ*rfe* Δ*typA ptsP::kan*	This work
RL254	W Δ*rfe* Δ*typA yobF::kan*	This work
RL255	W Δ*rfe* Δ*ptsP yobF::kan*	This work
RL256	W Δ*rfe* Δ*ptsP ackA::kan*	This work
RL257	W Δ*evgA* Δ*typA ptsP::kan*	This work
RL258	W Δ*evgA* Δ*typA yobF::kan*	This work
RL259	W Δ*evgA* Δ*typA ackA::kan*	This work
RL260	W Δ*evgA* Δ*typA yciW::kan*	This work
RL261	W Δ*evgA* Δ*ptsP yobF::kan*	This work
RL262	W Δ*evgA* Δ*ptsP yciW::kan*	This work
RL263	W Δ*typA* Δ*ptsP yobF::kan*	This work
RL264	W Δ*typA* Δ*ptsP ackA::kan*	This work
RL265	W Δ*typA* Δ*ptsP yciW::kan*	This work
RL266	W Δ*evgA* Δ*yciW yobF::kan*	This work
RL267	W Δ*evgA* Δ*yciW ackA::kan*	This work
RL268	W Δ*evgA* Δ*yciW ygaH::kan*	This work
RL269	W Δ*typA* Δ*yobF ackA::kan*	This work

RL270	W Δ*typA* Δ*yobF yciW::kan*	This work
RL271	W Δ*typA* Δ*yciW ackA::kan*	This work
RL272	W Δ*ptsP* Δ*yobF ackA::kan*	This work
RL273	W Δ*ptsP* Δ*yobF yciW::kan*	This work
RL274	W Δ*ptsP* Δ*yciW ackA::kan*	This work

1

Table S3. Numbers of larger colonies identified after selective plating of Tn libraries.

	selection condition	direct plating	serially passaged
W	0.6 M NaCl	2	5
	15 g/L NaAc	0	3
	5 g/L NaAc + 0.4 M NaCl	0	> 50
	pH 5.5 + 0.4 M NaCl	28	8
	pH 4.5	0	> 50
BL21(DE3)	0.6 M NaCl	12	0
	15 g/L NaAc	0	2
	5 g/L NaAc + 0.4 M NaCl	1	> 50
	pH 5.5 + 0.4 M NaCl	0	0
	pH 4.5	23	0

Table S4. Total number of Tn library selection isolates tested and the number of isolates identified as exhibiting qualitatively improved growth in liquid culture secondary screening.

	selection condition	isolates tested	isolates with improved growth	isolates tested	isolates with improved growth
W	0.6 M NaCl	2	2	5	3
	15 g/L NaAc	0	0	3	3
	5 g/L NaAc + 0.4 M NaCl	0	0	14	9
	pH 5.5 + 0.4 M NaCl	28	13	8	8
	pH 4.5	0	0	6	0
BL21(DE3)	0.6 M NaCl	12	1	0	0
	15 g/L NaAc	0	0	2	2
	5 g/L NaAc + 0.4 M NaCl	1	0	15	10
	pH 5.5 + 0.4 M NaCl	0	0	0	0
	pH 4.5	23	0	0	0

Table S5. Genes depleted in insertions in both W and BL21(DE3) following selection of Tn5 libraries in M9 + 0.5-0.6 M NaCl compared to selection in M9. Genes are only shown with adjusted $P < 0.05$ with the more conservative Bonferroni correction at top, and with the less conservative Benjamini Hochberg correction at bottom.

		W		BL21(DE3)	
gene	**description**	**fold change**	**P-value**	**fold change**	**P-value**
tolC	TolC outer membrane channel	-13.91	0.00	-2.13	0.00
rfaP	lipopolysaccharide core heptose (I) kinase	-12.90	0.00	-2.95	0.00
treR	TreR DNA-binding transcriptional repressor	-12.20	0.00	-10.40	0.00
otsB	trehalose-6-phosphate phosphatase	-11.80	0.00	-12.50	0.00
nadR	NadR DNA-binding trancriptional repressor and NMN adenylyltransferase	-11.75	0.00	-4.08	0.00
sbp	sulfate/thiosulfate ABC transporter, periplasmic binding protein Sbp	-10.97	0.00	-2.10	0.01
oppF	murein tripeptide/peptide ABC transporter, putative ATP binding subunit	-10.62	0.00	-8.23	0.00
prkB	predicted phosphoribulokinase	-10.57	0.00	-3.05	0.00
rfaG	lipopolysaccharide glucosyltransferase I	-9.94	0.00	-2.90	0.00
mrcA	peptidoglycan synthetase; penicillin-binding protein 1A	-9.28	0.00	-4.06	0.00
fepB	ferric enterobactin ABC transporter, periplasmic binding protein	-8.85	0.00	-5.02	0.00
lpoA	outer membrane lipoprotein – activator of PBP1A activity	-7.90	0.00	-5.01	0.00
clpA	ClpA ATP-dependent protease specificity component and chaperone	-7.79	0.00	-9.04	0.00
rseB	anti-sigma factor for sigma E	-7.68	0.00	-4.20	0.00
helD	DNA helicase IV	-7.55	0.00	-2.59	0.00
iclR	IclR (isocitrate lyase regulator) transcriptional repressor	-6.97	0.00	-8.54	0.00
mlaF	phospholipid ABC transporter, predicted ATP-binding component	-6.77	0.00	-23.52	0.00
mlaA	putative lipoprotein	-6.48	0.00	-15.41	0.00
mntR	component of MntR-Mn^{2+} DNA-binding transcriptional dual regulator	-5.22	0.00	-4.41	0.00
otsA	trehalose-6-phosphate synthase	-4.50	0.00	-24.26	0.00
ppk	polyphosphate kinase	-4.39	0.00	-5.85	0.00
pepA	aminopeptidase A/I and DNA-binding transcriptional repressor	-4.35	0.00	-3.18	0.01
fepC	ferric enterobactin ABC transporter, ATP binding subunit	-4.30	0.00	-3.49	0.00
mrcB	penicillin-binding protein 1B	-4.14	0.00	-4.82	0.00
cyoA	cytochrome *bo* terminal oxidase subunit II	-4.09	0.00	-24.55	0.00
yggT	predicted inner membrane protein	-3.94	0.00	-2.35	0.05
cpxA	CpxA sensory histidine kinase	-3.93	0.00	-4.08	0.00
uspE	universal stress protein with a role in cellular motility	-3.88	0.00	-4.68	0.00

rfaQ	lipopolysaccharide core heptosyl transferase III	-3.76	0.00	-1.87	0.01
dadA	D-amino acid dehydrogenase	-3.23	0.04	-4.66	0.00
rnr	RNase R	-3.17	0.00	-2.97	0.00
exbB	TonB energy transducing system, ExbB subunit	-12.55	0.00	-2.43	0.00
pgpA	phosphatidylglycerophosphatase A	-5.63	0.00	-2.41	0.04
galF	predicted uridylyltransferase subunit with GalU	-5.13	0.00	-2.15	0.01
fepG	ferric enterobactin ABC transporter, membrane subunit	-4.63	0.00	-13.01	0.00
fepD	ferric enterobactin ABC transporter, membrane subunit	-4.57	0.00	-7.25	0.00
cyoC	cytochrome *bo* terminal oxidase subunit III	-4.27	0.00	-9.86	0.00
yibN	predicted rhodanese-related sulfurtransferase	-4.17	0.00	-1.70	0.04
cyaA	adenylate cyclase	-3.96	0.00	-2.15	0.03
ahpC	alkyl hydroperoxide reductase, AhpC component	-3.89	0.01	-4.69	0.00
phoQ	PhoQ sensory histidine kinase	-3.74	0.00	-2.23	0.00
fbp	fructose-1,6-bisphosphatase I	-3.53	0.00	-1.58	0.02
tyrR	TyrR DNA-binding transcriptional dual regulator	-3.35	0.03	-7.39	0.00
rfaI	UDP-D-glucose:(glucosyl)LPS α-1,3-glucosyltransferase	-3.33	0.00	-1.57	0.01
yggS	predicted enzyme	-3.04	0.00	-1.81	0.03
envZ	EnvZ sensory histidine kinase	-2.75	0.00	-3.31	0.00
bioB	biotin synthase	-2.69	0.00	-1.64	0.01
recG	RecG DNA helicase	-2.56	0.00	-1.95	0.01
hslU	HslU hexamer component of HslVU protease	-2.44	0.00	-3.78	0.00
phoP	PhoP response regulator	-2.42	0.00	-2.63	0.00
dacA	D-alanyl-D-alanine carboxypeptidase IA; penicillin-binding protein 5	-2.28	0.00	-1.76	0.04
uspA	universal stress protein A	-2.21	0.00	-4.63	0.00
mlaC	phospholipid ABC transporter, predicted periplasmic binding protein	-2.20	0.04	-5.18	0.00
ysgA	predicted hydrolase	-2.20	0.00	-1.73	0.01
oppB	murein tripeptide /peptide ABC transporter, putative membrane subunit	-2.18	0.01	-19.06	0.00
tgt	tRNA-guanine transglycosylase	-2.09	0.04	-2.44	0.00
zntA	zinc, cadmium, and lead efflux system	-2.08	0.00	-6.35	0.00
mlaD	phospholipid ABC transporter, predicted substrate binding protein	-1.99	0.03	-19.18	0.00
mlaE	phospholipid ABC transporter, integral membrane component	-1.91	0.00	-4.23	0.00
maeB	malate dehydrogenase	-1.74	0.02	-1.54	0.03
dusA	tRNA-dihydrouridine synthase A	-1.73	0.01	-1.74	0.00
typA	protein possibly involved in ribosome structure or function	-1.67	0.01	-1.73	0.02
rapA	RNA polymerase (RNAP)-binding ATPase and RNAP recycling factor	-1.64	0.01	-3.70	0.00
acs	acetyl-CoA synthetase (AMP-forming)	-1.64	0.02	-2.64	0.00
fabR	FabR DNA-binding transcriptional repressor	-1.59	0.03	-3.04	0.00

Table S6. Genes depleted in insertions only strain W and not in strain BL21(DE3) following selection of Tn5 libraries in M9 + 0.5-0.6 M NaCl compared to selection in M9. Genes are only shown with adjusted $P < 0.05$ with the Benjamini Hochberg correction.

locus[1]	gene	description	fold-change	adj. P-value
b4000	hupA	transcriptional dual regulator HU-α (HU-2)	-16.12	0.00
b3459	yhhK	maturation factor for PanD	-13.01	0.00
b2912	fau	5-formyltetrahydrofolate cyclo-ligase	-12.32	0.00
b1235	rssB	regulator of RpoS	-11.92	0.00
b0080	cra	Cra DNA-binding transcriptional dual regulator	-10.39	0.00
b3205	rapZ	RNase adaptor protein	-10.00	0.00
b3650	spoT	guanosine 3'-diphosphate 5'-triphosphate 3'-diphosphatase	-8.83	0.00
b0839	dacC	penicillin-binding protein 6	-8.69	0.00
b0441	ppiD	periplasmic folding helper protein	-8.24	0.00
b2671	ygaC	predicted protein	-7.92	0.00
b0688	pgm	phosphoglucomutase	-7.62	0.00
b0584	fepA	ferric enterobactin/colicin B/colicin D outer membrane porin FepA	-7.40	0.00
b3191	mlaB	MlaB subunit of phospholipid ABC transporter	-7.33	0.00
b3641	slmA	cell division inhibitor, nucleoid occlusion	-7.20	0.00
b1682	sufC	SufC component of SufBCD Fe-S cluster scaffold complex	-7.19	0.00
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase	-6.87	0.00
b3212	gltB	glutamate synthase, large subunit	-6.69	0.00
b2897	sdhE	FAD assembly factor	-6.01	0.00
b2697	alaS	alanyl-tRNA synthetase and DNA-binding transcriptional repressor	-5.68	0.00
b3842	rfaH	RfaH transcriptional antiterminator	-5.66	0.00
b3032	cpdA	cAMP phosphodiesterase	-5.47	0.00
b4401	ptsG	glucose PTS permease - PtsG subunit	-5.46	0.00
b1232	purU	formyltetrahydrofolate deformylase	-5.18	0.00
b3363	ppiA	peptidyl-prolyl *cis-trans* isomerase A (rotamase A)	-5.11	0.00
b1049	opgH	membrane glycosyltransferase; synthesis of osmoregulated periplasmic glucans (OPGs)	-5.05	0.00
b2822	recC	RecC subunit of RecBCD helicase/nuclease (exonuclease V) complex	-4.67	0.00
b0460	hha	haemolysin expression modulating protein	-4.42	0.00
b2837	galR	GalR DNA-binding transcriptional dual regulator	-4.40	0.00
b4217	ytfK	conserved protein	-4.34	0.00
b2494	bepA	chaperone/protease involved in the	-4.32	0.00

		maintenance of OM integrity		
b0390	aroM	conserved protein	-4.30	0.00
b3940	metL	aspartate kinase / homoserine dehydrogenase	-4.30	0.00
b1784	yeaH	conserved protein	-4.27	0.00
b3349	slyD	FKBP-type peptidyl prolyl *cis-trans* isomerase	-4.16	0.01
b2215	ompC	outer membrane porin C	-4.03	0.00
b3688	yidQ	conserved outer membrane protein	-3.91	0.00
b1090	plsX	fatty acid/phospholipid synthesis protein	-3.89	0.01
ECW_m1499	ECW_m1499	hypothetical protein	-3.73	0.01
b3849	trkH	K^+ transporter TrkH	-3.71	0.00
ECW_m0342	lafE	flagellar hook-length control protein	-3.61	0.00
b2502	ppx	exopolyphosphatase	-3.57	0.00
b3252	csrD	regulator of CsrB and CsrC decay	-3.49	0.00
b3213	gltD	glutamate synthase, small subunit	-3.45	0.00
b4046	zur	DNA-binding transcriptional repressor, Zn(II)-binding protein	-3.44	0.00
b1859	znuB	Zn^{2+} ABC transporter - membrane subunit	-3.39	0.03
b1706	ydiU	conserved protein	-3.33	0.00
b2436	hemF	coproporphyrinogen III oxidase	-3.30	0.00
b1286	rnb	ribonuclease III	-3.27	0.00
b0425	panE	2-dehydropantoate 2-reductase	-3.27	0.00
b3350	kefB	potassium:proton antiporter	-3.23	0.00
b1188	ycgB	conserved protein	-3.15	0.00
b1598	ydgD	predicted peptidase	-3.15	0.00
b0767	pgl	6-phosphogluconolactonase	-3.14	0.00
b1711	btuC	vitamin B12 ABC transporter - membrane subunit	-3.13	0.03
b0463	acrA	membrane fusion protein subunit of AcrAD-TolC multidrug efflux transport system	-3.06	0.00
b1641	slyB	outer membrane lipoprotein	-3.05	0.00
b3336	bfr	bacterioferritin monomer	-2.97	0.00
b4402	yjjY	predicted protein	-2.95	0.03
b3232	yhcM	conserved protein with a nucleoside triphosphate hydrolase domain	-2.93	0.00
b0102	zapD	cell division factor ZapD	-2.89	0.00
b3055	ygiM	predicted signal transduction protein (SH3 domain)	-2.86	0.00
b4035	malK	maltose ABC transporter - ATP binding subunit	-2.84	0.00
b0581	ybdK	carboxylate-amine ligase	-2.82	0.00
b3168	infB	protein chain initiation factor IF2	-2.80	0.04
b0240	crl	RNA polymerase holoenzyme assembly factor Crl	-2.76	0.00

b3351	kefG	protein required for KefB activity	-2.65	0.00
b0426	yajQ	nucleotide binding protein	-2.60	0.00
b0214	lon	DNA-binding, ATP-dependent protease La	-2.51	0.04
b2808	gcvA	GcvA DNA-binding transcriptional dual regulator	-2.47	0.01
b3813	rpoB	RNA polymerase, β subunit	-2.45	0.03
b4242	mgtA	$Mg2+/Ni^{2+}$ transporting ATPase	-2.42	0.00
b2895	fldB	flavodoxin 2	-2.42	0.03
b0832	gsiD	glutathione ABC transporter - membrane subunit	-2.40	0.00
b0440	hupB	transcriptional dual regulator HU-β, NS1 (HU-1)	-2.40	0.00
b0857	potI	putrescine ABC transporter - membrane subunit	-2.39	0.00
b1683	sufB	SufB component of SufBCD Fe-S cluster scaffold complex	-2.38	0.02
b3031	yqiA	esterase	-2.38	0.00
b3779	gpp	guanosine 5'-triphosphate, 3'-diphosphate pyrophosphatase	-2.36	0.00
b0840	deoR	DeoR DNA-binding transcriptional repressor	-2.35	0.00
b2749	ygbE	conserved inner membrane protein	-2.34	0.00
b4258	valS	valyl-tRNA synthetase	-2.34	0.03
b0829	gsiA	glutathione ABC transporter - ATP binding subunit	-2.33	0.00
b0026	ileS	isoleucyl-tRNA synthetase	-2.31	0.01
b2470	acrD	permease subunit of AcrAD-TolC multidrug efflux transport system	-2.31	0.02
b1783	yeaG	protein kinase	-2.31	0.02
b4679	yohP	small membrane protein	-2.31	0.03
b0831	gsiC	glutathione ABC transporter - membrane subunit	-2.29	0.01
b2790	yqcA	predicted flavoprotein	-2.22	0.01
b0427	yajR	YajR MFS transporter	-2.22	0.00
b3625	rfaY	lipopolysaccharide core heptose (II) kinase	-2.21	0.00
b1816	yoaE	predicted inner membrane protein	-2.17	0.04
b2029	gnd	6-phosphogluconate dehydrogenase (decarboxylating)	-2.17	0.00
b1113	ycfS	L,D-transpeptidase	-2.14	0.01
b2464	talA	transaldolase A	-2.11	0.01
b2587	kgtP	α-ketoglutarate:H^+ symporter	-2.11	0.00
b1819	manZ	mannose PTS permease - ManZ subunit	-2.09	0.01
b2829	ptsP	phosphoenolpyruvate-protein phosphotransferase PtsP, enzyme I^{Ntr}	-2.02	0.01
b1976	mtfA	Mlc titration factor	-1.99	0.01
ECW_m0335	lfgL	flagellar hook-filament junction protein	-1.97	0.01
b1943	fliK	flagellar hook-length control protein	-1.92	0.03

b0434	yajG	predicted lipoprotein	-1.91	0.03
b1560	ydfU	Qin prophage; predicted protein	-1.88	0.04
b2592	clpB	ClpB chaperone	-1.86	0.01
b0393	rdgC	nucleoid-associated protein RdgC	-1.84	0.04
b4504	ykfH	predicted protein	-1.84	0.00
b2465	tktB	transketolase II	-1.83	0.03
b1492	gadC	glutamic acid:4-aminobutyrate antiporter	-1.82	0.01
b4207	fklB	FKBP-type peptidyl prolyl cis-trans isomerase (rotamase)	-1.79	0.00
b0820	ybiT	putative ATP-binding component of a transport system	-1.79	0.02
b1190	dadX	alanine racemase 2, PLP-binding	-1.78	0.04
b0800	ybiB	predicted transferase/phosphorylase	-1.77	0.04
b1817	manX	mannose PTS permease - ManX subunit	-1.76	0.03
ECW_m0922	ECW_m0922	terminase, ATPase subunit	-1.76	0.01
b1604	ydgH	predicted protein	-1.75	0.05
b1075	flgD	flagellar biosynthesis, initiation of hook assembly	-1.71	0.01
b0212	gloB	glyoxalase II	-1.71	0.03
b0493	ybbO	predicted oxidoreductase with NAD(P)-binding Rossmann-fold domain	-1.69	0.04
b3810	yigA	conserved protein	-1.69	0.03
b3740	rsmG	16S rRNA m^7G527 methyltransferase	-1.66	0.00
b4180	rlmB	23S rRNA 2'-O-ribose G2251 methyltransferase monomer	-1.65	0.03
b2014	plaP	putrescine:H^+ symporter	-1.65	0.03
ECW_m3904	waaT	UDP-galactose:(glucosyl) LPS a1,2-galactosyltransferase	-1.62	0.00
b2106	rcnA	membrane protein conferring nickel and cobalt resistance	-1.61	0.04
b2736	ygbJ	predicted dehydrogenase, with NAD(P)-binding Rossmann-fold domain	-1.59	0.01
b2835	lplT	lysophospholipid transporter	-1.57	0.03
ECW_m1506	ECW_m1506	side tail fiber protein	-1.56	0.02
b2257	arnT	4-amino-4-deoxy-L-arabinose (L-Ara4N) transferase	-1.56	0.04
b1660	ydhC	predicted transport protein	-1.55	0.02
b3755	yieP	predicted transcriptional regulator	-1.53	0.04
b0598	cstA	peptide transporter induced by carbon starvation	-1.49	0.04
b4389	radA	DNA recombination protein	-1.47	0.04

[1] locus tags are for the corresponding genes in K-12 MG1655, except when the gene does not exist in K-12 MG1655. The W locus tag is used in these cases.

Table S7. Genes depleted in insertions only strain BL21(DE3) and not in strain W following selection of Tn5 libraries in M9 + 0.5-0.6 M NaCl compared to selection in M9. Genes are only shown with adjusted $P < 0.05$ with the Benjamini Hochberg correction.

locus[1]	gene	description	fold-change	adj P-value
b1748	astC	succinylornithine transaminase, PLP-dependent	-26.73	0.00
b0728	sucC	succinyl-CoA synthetase subunit beta	-25.44	0.00
b1243	oppA	oligopeptide transporter subunit	-19.41	0.00
b3437	gntK	gluconate kinase	-18.54	0.00
b3438	gntR	DNA-binding transcriptional repressor	-15.24	0.00
b1823	cspC	stress protein, member of the CspA-family	-12.51	0.00
b1744	astE	succinylglutamate desuccinylase	-10.53	0.00
b2168	fruK	1-phosphofructokinase	-10.04	0.00
b1423	ydcJ	hypothetical protein	-9.17	0.00
b1246	oppD	oligopeptide ABC transporter ATP-binding protein	-8.73	0.00
b0428	cyoE	protoheme IX farnesyltransferase	-8.72	0.00
b3790	rffC	TDP-fucosamine acetyltransferase	-8.39	0.00
b1746	astD	succinylglutamic semialdehyde dehydrogenase	-8.36	0.00
b4175	hflC	modulator for HflB protease specific for phage lambda cII repressor	-8.23	0.00
b0221	fadE	acyl-CoA dehydrogenase	-8.03	0.00
b3423	glpR	DNA-binding transcriptional repressor	-7.65	0.00
b4395	ytjC	phosphoglycerate mutase	-7.43	0.00
b0526	cysS	cysteinyl-tRNA synthetase	-7.23	0.00
b2425	cysP	thiosulfate transporter subunit	-7.20	0.00
ECD_04231	hpaR	Homoprotocatechuate degradative operon repressor	-7.10	0.00
b1856	yebA	peptidase	-6.92	0.00
b4205	ytfA	transcriptional regulator	-6.53	0.00
b1205	ychH	inner membrane protein	-6.42	0.00
b1745	astB	succinylarginine dihydrolase	-6.07	0.00
b1824	yobF	hypothetical protein	-6.02	0.00
b3724	phoU	negative regulator of PhoR/PhoB two-component regulator	-5.98	0.00
b3228	sspB	ClpXP protease specificity-enhancing factor	-5.83	0.00
b1747	astA	arginine succinyltransferase	-5.67	0.00
b2684	mprA	DNA-binding transcriptional repressor	-5.61	0.00
b2114	metG	methionyl-tRNA synthetase	-5.59	0.00
b2700	ygaD	competence damage-inducible protein A	-5.42	0.00
b0585	fes	enterobactin/ferric enterobactin esterase	-5.42	0.00

b1105	lpoB	outer membrane lipoprotein	-5.42	0.00
b1103	hinT	purine nucleoside phosphoramidase	-5.28	0.00
b3791	rffA	TDP-4-oxo-6-deoxy-D-glucose transaminase	-5.23	0.00
b3494	uspB	universal stress protein UspB	-5.22	0.00
b1014	putA	multifunctional DNA-binding transcriptional regulator/proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase	-5.14	0.00
b2688	gshA	glutamate-cysteine ligase	-4.99	0.00
b0755	gpmA	phosphoglyceromutase	-4.96	0.00
b3464	ftsY	fused Signal Recognition Particle (SRP) receptor: membrane binding protein/hypothetical protein	-4.79	0.02
b0049	apaH	diadenosine tetraphosphatase	-4.75	0.00
b3860	dsbA	protein disulfide isomerase I	-4.60	0.00
b1252	tonB	membrane spanning protein in TonB-ExbB-ExbD complex	-4.57	0.00
b3984	rplA	50S ribosomal protein L1	-4.50	0.03
b0734	cydB	cytochrome d terminal oxidase, subunit II	-4.48	0.01
b0729	sucD	succinyl-CoA synthetase subunit alpha	-4.45	0.00
b4174	hflK	modulator for HflB protease specific for phage lambda cII repressor	-4.39	0.00
b2283	nuoG	NADH dehydrogenase subunit G	-4.36	0.00
b3203	hpf	ribosome-associated, sigma 54 modulation protein	-4.30	0.00
b1284	yciT	DNA-binding transcriptional regulator	-4.28	0.00
b2412	zipA	cell division protein ZipA	-4.20	0.05
b1095	fabF	3-oxoacyl-(acyl carrier protein) synthase	-4.19	0.01
b1249	cls	cardiolipin synthetase	-4.15	0.00
b1854	pykA	pyruvate kinase	-4.12	0.00
b3178	ftsH	protease, ATP-dependent zinc-metalloprotease	-4.07	0.00
b0846	ybjK	DNA-binding transcriptional regulator	-3.98	0.00
b2947	gshB	glutathione synthetase	-3.98	0.00
b0436	tig	trigger factor	-3.98	0.00
b0636	rlmH	hypothetical protein	-3.96	0.02
b1438	hicB	DNA-binding transcriptional regulator	-3.93	0.00
b2742	nlpD	outer membrane lipoprotein	-3.87	0.00
b0431	cyoB	cytochrome o ubiquinol oxidase subunit I	-3.85	0.00
b1136	icd	isocitrate dehydrogenase	-3.82	0.02
b2308	hisQ	histidine/lysine/arginine/ornithine transporter subunit	-3.81	0.00
b3932	hslV	ATP-dependent protease peptidase subunit	-3.77	0.00
b2299	yfcD	NUDIX hydrolase	-3.68	0.00
b1276	acnA	aconitate hydratase	-3.67	0.00
b1968	yedV	sensory kinase in two-component regulatory	-3.66	0.00

		system with YedW		
b0369	hemB	delta-aminolevulinic acid dehydratase	-3.64	0.02
b4020	yjbB	transporter	-3.60	0.00
b1630	rsxD	electron transport complex protein RnfD	-3.58	0.00
b1761	gdhA	glutamate dehydrogenase	-3.56	0.00
b1857	znuA	high-affinity zinc transporter periplasmic protein	-3.54	0.00
b1087	yceF	Maf-like protein	-3.53	0.00
b4244	pyrI	aspartate carbamoyltransferase regulatory subunit	-3.53	0.00
b4191	ulaR	DNA-binding transcriptional dual regulator	-3.48	0.00
b1830	prc	carboxy-terminal protease for penicillin-binding protein 3	-3.48	0.00
b2306	hisP	histidine/lysine/arginine/ornithine transporter subunit	-3.47	0.00
b0177	bamA	hypothetical protein	-3.45	0.05
b1627	rsxA	Na(+)-translocating NADH-quinone reductase subunit E	-3.41	0.01
b3162	deaD	ATP-dependent RNA helicase	-3.36	0.00
b3792	wzxE	O-antigen translocase	-3.35	0.00
b0676	nagC	DNA-binding transcriptional dual regulator	-3.34	0.00
b2307	hisM	histidine/lysine/arginine/ornithine transporter subunit	-3.33	0.00
b4173	hflX	GTPase	-3.33	0.00
b3304	rplR	50S ribosomal protein L18	-3.29	0.00
b1092	fabD	acyl carrier protein S-malonyltransferase	-3.23	0.01
b2553	glnB	regulatory protein P-II for glutaminesynthetase	-3.23	0.01
b3574	yiaJ	DNA-binding transcriptional repressor	-3.22	0.00
b2504	yfgG	hypothetical protein	-3.18	0.01
ECD_01690	ECD_01690	hypothetical protein	-3.17	0.00
b2309	hisJ	histidine/lysine/arginine/ornithine transporter subunit	-3.15	0.00
b4484	cpxP	periplasmic protein combats stress	-3.11	0.00
b3784	rfe	UDP-GlcNAc:undecaprenylphosphate GlcNAc-1-phosphate transferase	-3.08	0.00
b1097	yceG	aminodeoxychorismate lyase	-3.06	0.01
ECD_10023	cII	antitermination protein	-3.00	0.00
b1594	dgsA	DNA-binding transcriptional repressor	-2.98	0.02
b1713	pheT	phenylalanyl-tRNA synthetase subunit beta	-2.96	0.03
b2817	amiC	N-acetylmuramoyl-L-alanine amidase	-2.94	0.00
b3783	uvrD	DNA-dependent ATPase I and helicase II	-2.93	0.01
b3780	rhlB	ATP-dependent RNA helicase	-2.88	0.00
b3794	rffM	UDP-N-acetyl-D-mannosaminuronic acid transferase	-2.86	0.00

b3247	rng	ribonuclease G	-2.85	0.01
b0020	nhaR	DNA-binding transcriptional activator	-2.82	0.00
b1596	ynfM	transporter	-2.80	0.00
b0113	pdhR	pyruvate dehydrogenase complex transcriptional regulator	-2.77	0.05
b3787	rffD	UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase	-2.76	0.00
b0464	acrR	DNA-binding transcriptional repressor	-2.75	0.00
b3260	dusB	tRNA-dihydrouridine synthase B	-2.67	0.01
b4210	ytfF	inner membrane protein	-2.65	0.00
b2751	cysN	sulfate adenylyltransferase subunit 1	-2.64	0.01
b1488	ddpX	D-Ala-D-Ala dipeptidase	-2.63	0.01
b3500	gor	glutathione reductase	-2.62	0.00
b3846	fadB	bifunctional 3-hydroxybutyryl-CoA epimerase/delta(3)-cis-delta(2)-trans-enoyl-CoA isomerase/enoyl-CoA hydratase/ 3-hydroxyacyl-CoA dehydrogenase	-2.59	0.00
b3465	rsmD	methyltransferase	-2.58	0.02
b3655	yicH	hypothetical protein	-2.55	0.00
b4383	deoB	phosphopentomutase	-2.55	0.00
b3786	rffE	UDP-N-acetyl glucosamine-2-epimerase	-2.54	0.00
b1255	yciC	hypothetical protein	-2.50	0.02
b4251	yjgJ	transcriptional regulator	-2.48	0.00
b3643	rph	ribonuclease PH	-2.48	0.00
b3912	cpxR	DNA-binding response regulator in two-component regulatory system with CpxA	-2.48	0.00
b2344	fadL	long-chain fatty acid outer membrane transporter	-2.46	0.00
b2007	yeeX	hypothetical protein	-2.44	0.00
b2287	nuoB	NADH dehydrogenase subunit B	-2.44	0.02
b2418	pdxK	pyridoxine kinase	-2.42	0.00
b3956	ppc	phosphoenolpyruvate carboxylase	-2.42	0.03
b1164	ycgZ	hypothetical protein	-2.39	0.04
b3888	yiiD	acetyltransferase	-2.37	0.00
b2516	rodZ	hypothetical protein	-2.36	0.00
b3053	glnE	bifunctional deadenylyltransferase/adenylyltransferase	-2.35	0.00
b4208	cycA	D-alanine/D-serine/glycine transporter	-2.31	0.00
b2289	lrhA	DNA-binding transcriptional repressor of flagellar, motility and chemotaxis genes	-2.29	0.00
b1132	hflD	hypothetical protein	-2.23	0.04
b2310	argT	lysine/arginine/ornithine transporter subunit	-2.22	0.00
b1687	ydiJ	FAD-linked oxidoreductase	-2.17	0.03

b1381	ydbH	hypothetical protein	-2.12	0.00
b1123	potD	spermidine/putrescine ABC transporter substrate-binding protein	-2.07	0.00
b1704	aroH	3-deoxy-D-arabinoheptulosonate-7-phosphatesynthase	-2.06	0.00
b1805	fadD	acyl-CoA synthase	-2.04	0.00
b3005	exbD	membrane spanning protein in TonB-ExbB-ExbD complex	-2.02	0.00
b0197	metQ	DL-methionine transporter subunit	-2.02	0.01
b3816	corA	magnesium/nickel/cobalt transporter	-2.01	0.02
b1114	mfd	transcription-repair coupling factor	-1.98	0.00
b1499	ydeO	DNA-binding transcriptional acfivator	-1.96	0.02
ECD_01416	yhhI_3	hypothetical protein	-1.95	0.05
ECD_00840	ECD_00840	hypothetical protein	-1.94	0.02
b1767	ansA	asparaginase	-1.92	0.00
b1180	ycgM	isomerase/hydrolase	-1.91	0.02
b3847	pepQ	proline dipeptidase	-1.91	0.00
b2295	yfbV	hypothetical protein	-1.90	0.04
b3673	emrD	multidrug efflux system protein	-1.90	0.00
b0469	apt	adenine phosphoribosyltransferase	-1.89	0.00
b3054	ygiF	adenylate cyclase	-1.85	0.00
b3146	rsmI	methyltransferase	-1.83	0.00
b2144	sanA	hypothetical protein	-1.83	0.01
b3908	sodA	superoxide dismutase	-1.79	0.00
b3529	yhjK	diguanylate cyclase	-1.79	0.00
b3026	qseC	sensory histidine kinase in two-component regulatory system with QseB	-1.78	0.00
b1725	yniA	phosphotransferase/kinase	-1.77	0.04
b3920	yiiQ	hypothetical protein	-1.76	0.01
b1422	ydcI	DNA-binding transcriptional regulator	-1.75	0.04
b0752	zitB	zinc transporter ZitB	-1.73	0.03
b2055	wcaE	glycosyl transferase family protein	-1.72	0.01
b3653	gltS	glutamate transporter	-1.72	0.01
b1040	csgD	DNA-binding transcriptional activator in two-component regulatory system	-1.71	0.01
b0473	htpG	heat shock protein 90	-1.70	0.00
b4292	fecR	transmembrane signal transducer for ferric citrate transport	-1.69	0.05
b0861	artM	arginine transporter subunit	-1.69	0.01
b3616	tdh	L-threonine 3-dehydrogenase	-1.68	0.02
b1576	ydfD	hypothetical protein	-1.68	0.02
b4391	yjjK	sugar ABC transporter ATP-binding protein	-1.67	0.03
b3591	selA	selenocysteine synthase	-1.67	0.03

b3473	yhhS	transporter	-1.67	0.04
b3347	fkpA	FKBP-type peptidyl-prolyl cis-trans isomerase	-1.67	0.00
b3961	oxyR	DNA-binding transcriptional dual regulator	-1.67	0.03
b1365	ynaK	hypothetical protein	-1.63	0.00
b0461	tomB	hypothetical protein	-1.63	0.01
b3541	dppD	dipeptide transporter	-1.62	0.00
b1782	mipA	scaffolding protein for murein synthesizing machinery	-1.62	0.01
b3617	kbl	2-amino-3-ketobutyrate coenzyme A ligase	-1.62	0.02
b2659	csiD	hypothetical protein	-1.61	0.01
b2163	yeiL	DNA-binding transcriptional activator	-1.59	0.01
b3253	yhdH	oxidoreductase, Zn-dependent and NAD(P)-binding	-1.59	0.04
b1535	ydeH	hypothetical protein	-1.59	0.00
ECD_02649	ECD_02649	L-ribulokinase AraB-like protein	-1.58	0.02
b1691	ydiN	transporter	-1.56	0.00
b1250	kch	voltage-gated potassium channel	-1.55	0.01
b2584	yfiQ	acyl-CoA synthetase NAD(P)-binding subunit/ATP-binding subunit	-1.55	0.03
b3354	yheU	hypothetical protein	-1.55	0.02
ECD_02511	ECD_02511	ParB family protein	-1.54	0.04
b3789	rffH	glucose-1-phosphate thymidylyltransferase	-1.54	0.02
b2369	evgA	DNA-binding response regulator in two-component regulatory system with EvgS	-1.53	0.04
ECD_03522	ssb_2	ssDNA-binding protein	-1.53	0.03
ECD_01506	ECD_01506	hypothetical protein	-1.52	0.04
b1694	ydiF	acetyl-CoA:acetoacetyl-CoA transferase alpha and beta subunits	-1.51	0.02
b0449	mdlB	multidrug ABC transporter ATP-binding protein	-1.47	0.03
b2372	yfdV	transporter	-1.41	0.04

[1]locus tags are for the corresponding genes in K-12 MG1655, except when the gene does not exist in K-12 MG1655. The BL21(DE3) locus tag is used in these cases.

1

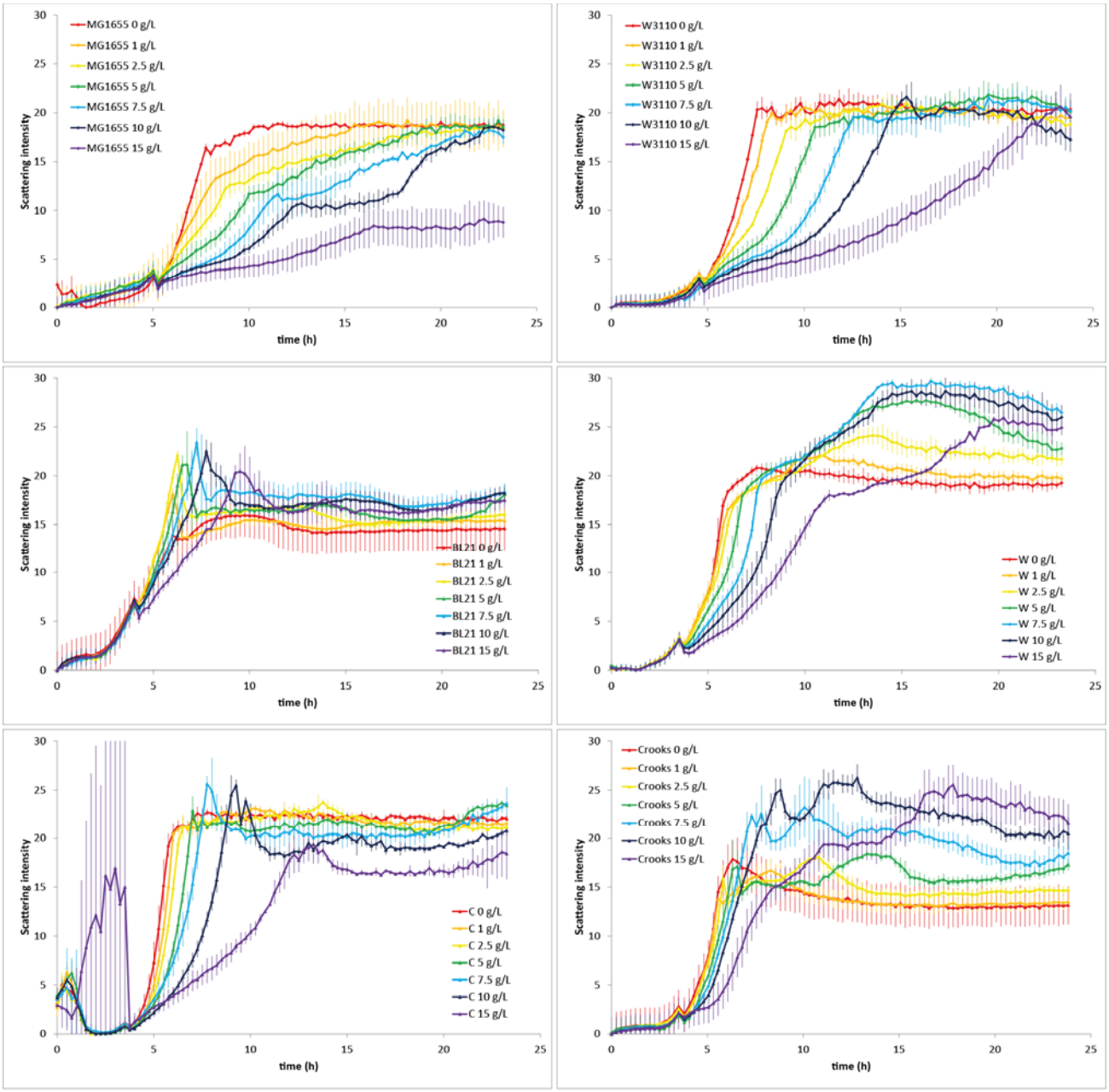


Fig. S1. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) grown with a concentration series of sodium acetate between 0 and 15 g/L. Sodium acetate was added during exponential growth (5 h for MG1655 and W3110; 4 h for BL21(DE3); 3.5 h for W, C, and Crooks).

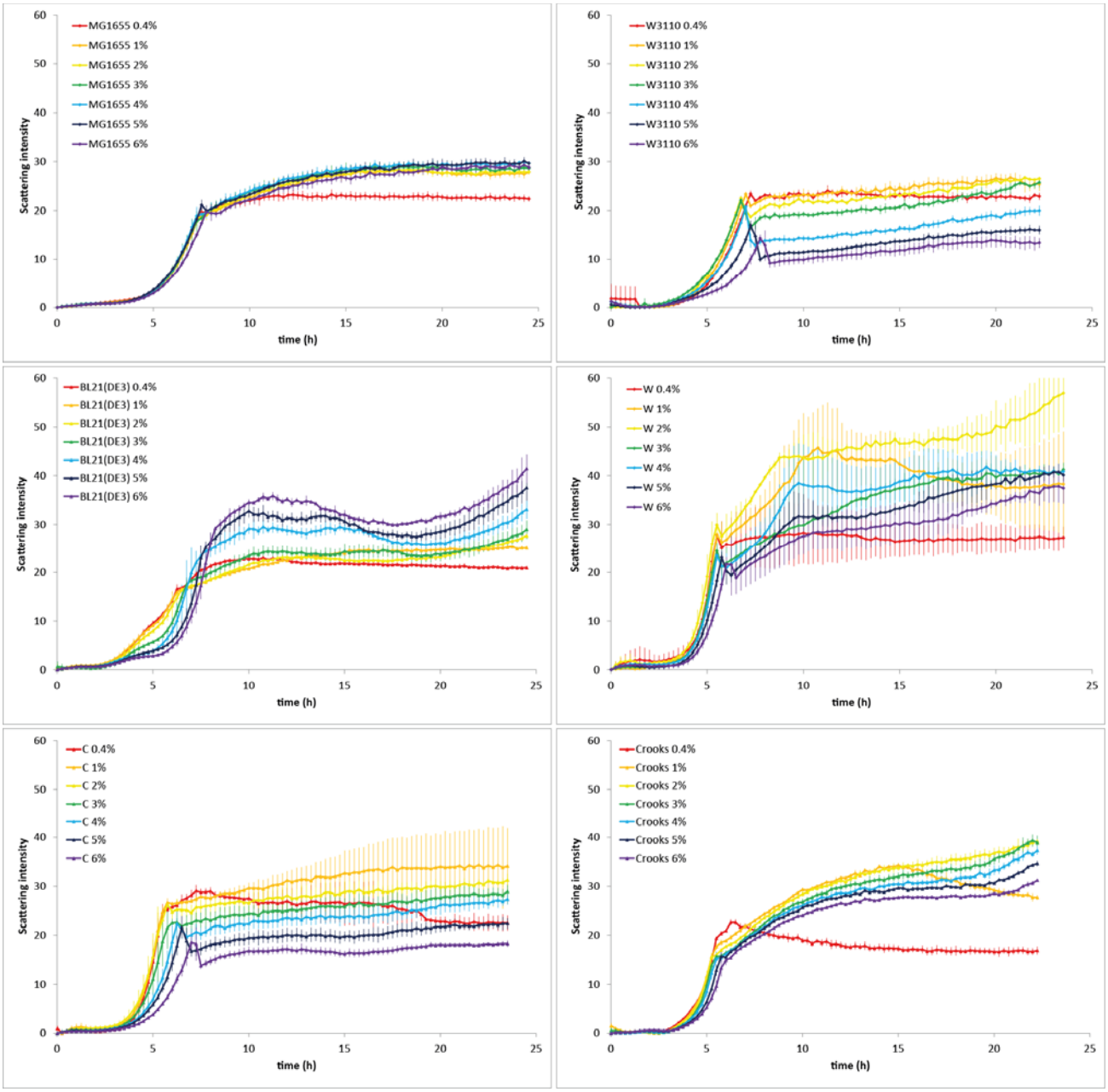


Fig. S2. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) grown with a concentration series of glucose between 0.4% and 6% w/v. The indicated glucose concentration was present in the culture medium from the time of inoculation.

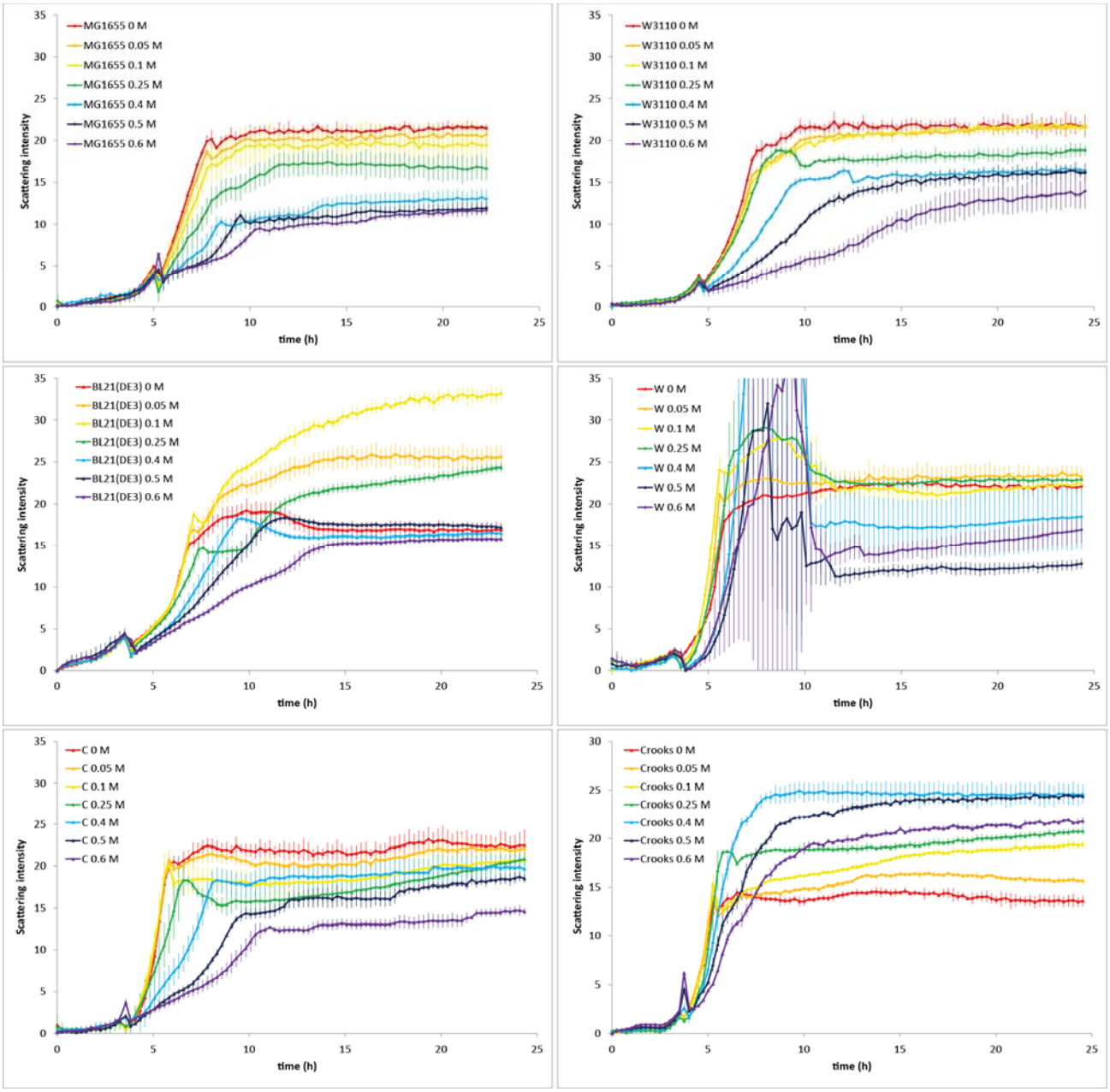


Fig. S3. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) grown with a concentration series of NaCl (in addition to that present already in M9 medium) between 0 M and 0.6 M. NaCl was added during exponential growth (5 h for MG1655; 4.5 h for W3110; 3.5 h for BL21(DE3), W, C, and Crooks).

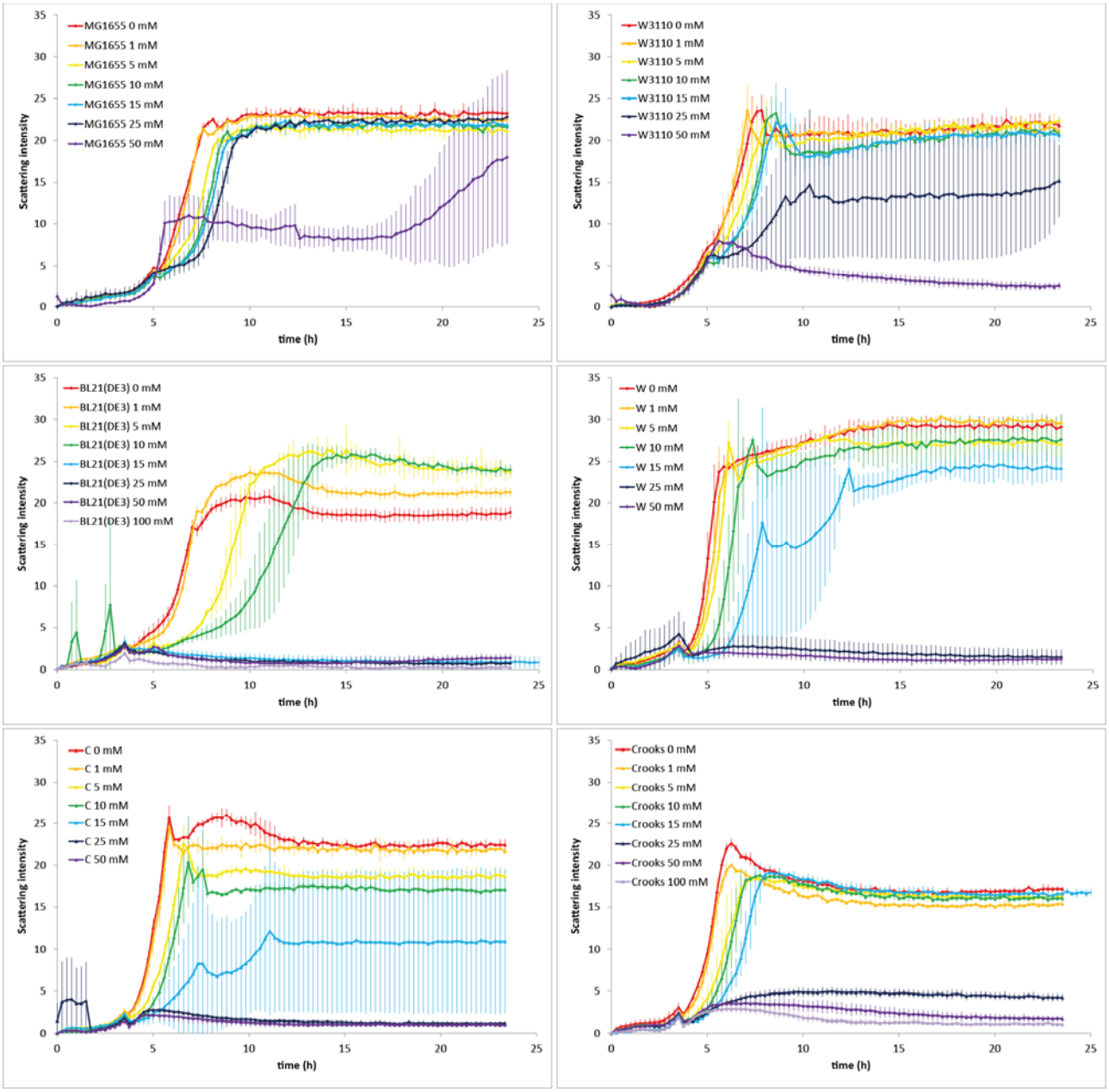


Fig. S4. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) grown with a concentration series of H_2O_2 between 0 and 100 mM. H_2O_2 was added during exponential growth (5 h for MG1655 and W3110; 3.5 h for BL21(DE3), W, C, and Crooks).

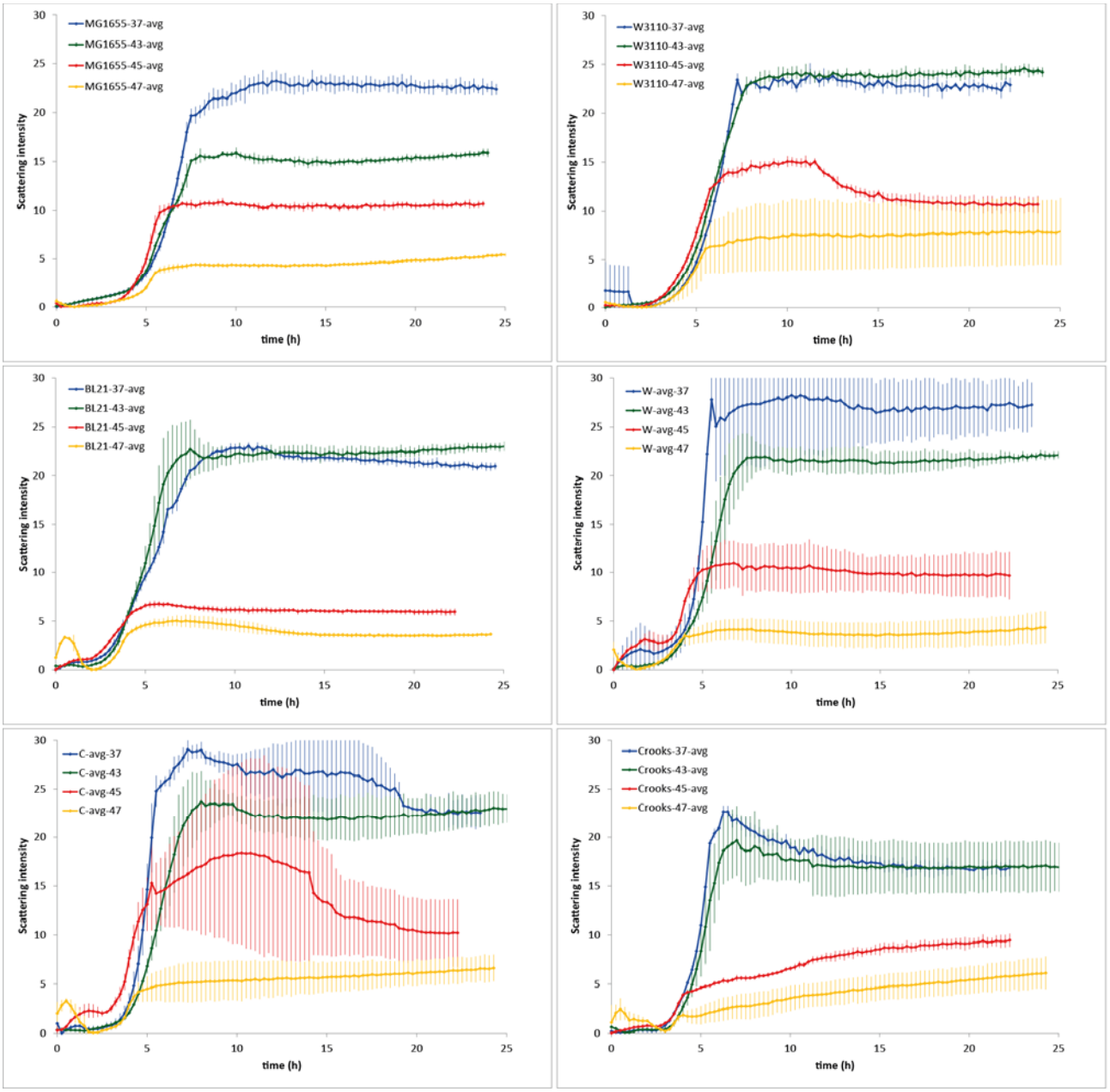


Fig. S5. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) cultivated at 37°C with step increases in temperature applied during exponential growth (5 h for MG1655 and W3110; 3.5 h for BL21(DE3), W, C, and Crooks).

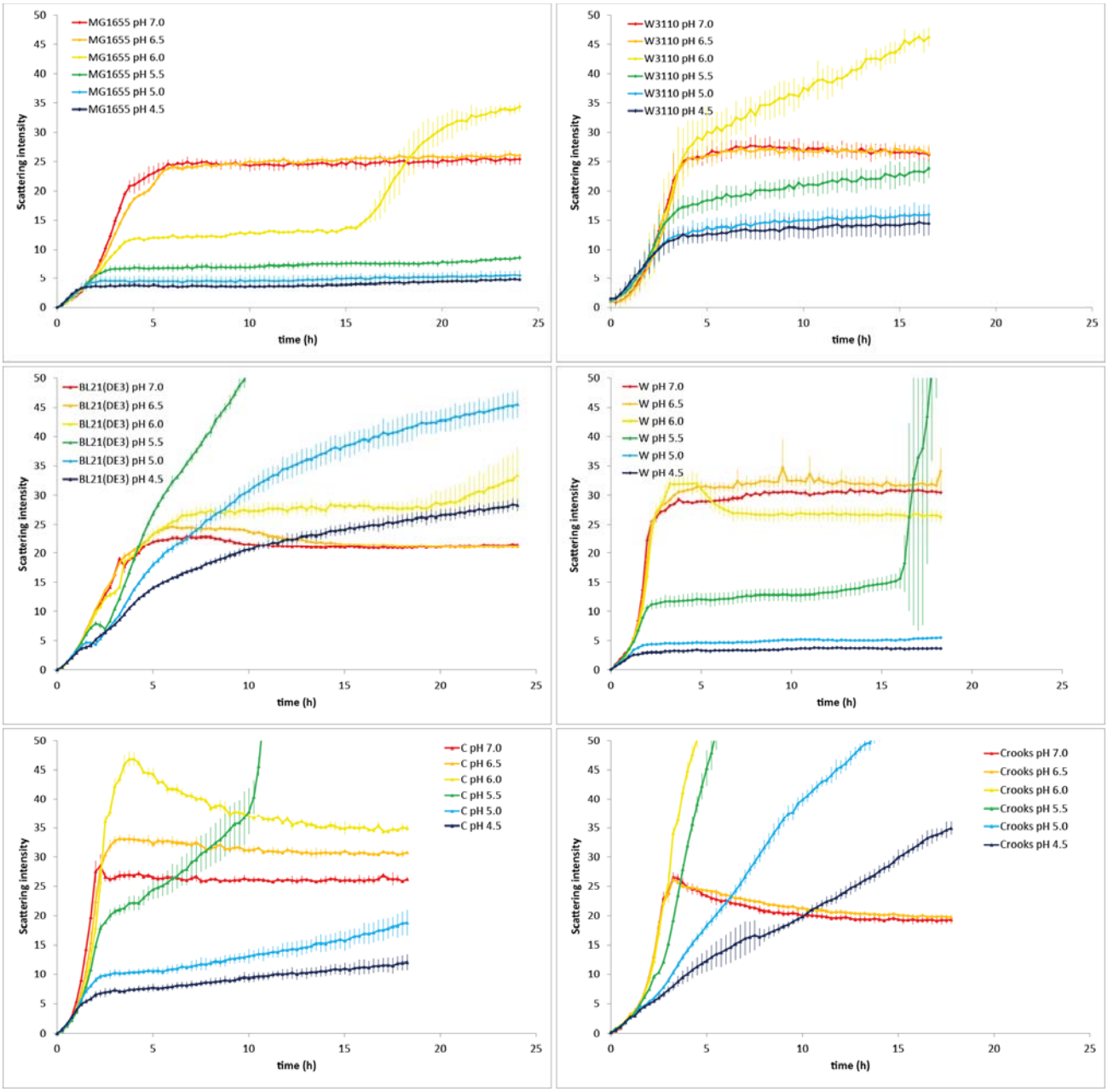


Fig. S6. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) cultivated in M9 pH 7.0 until mid-exponential growth and subsequently transferred into M9 adjusted to different pH values (transfers were performed when the following OD_{600} values were measured for each strains: 1.0 for K-12 MG1655, 1.1-1.6 for K-12 W3110, 0.55 for BL21(DE3), 1.1-1.9 for W, 1.0-1.1 for C, and 1.5-1.6 for

Crooks). Online growth monitoring began after transferring cells to the media with varying pH values, with the initial scattering intensity subtracted out from all later scattering intensity values in each plot.

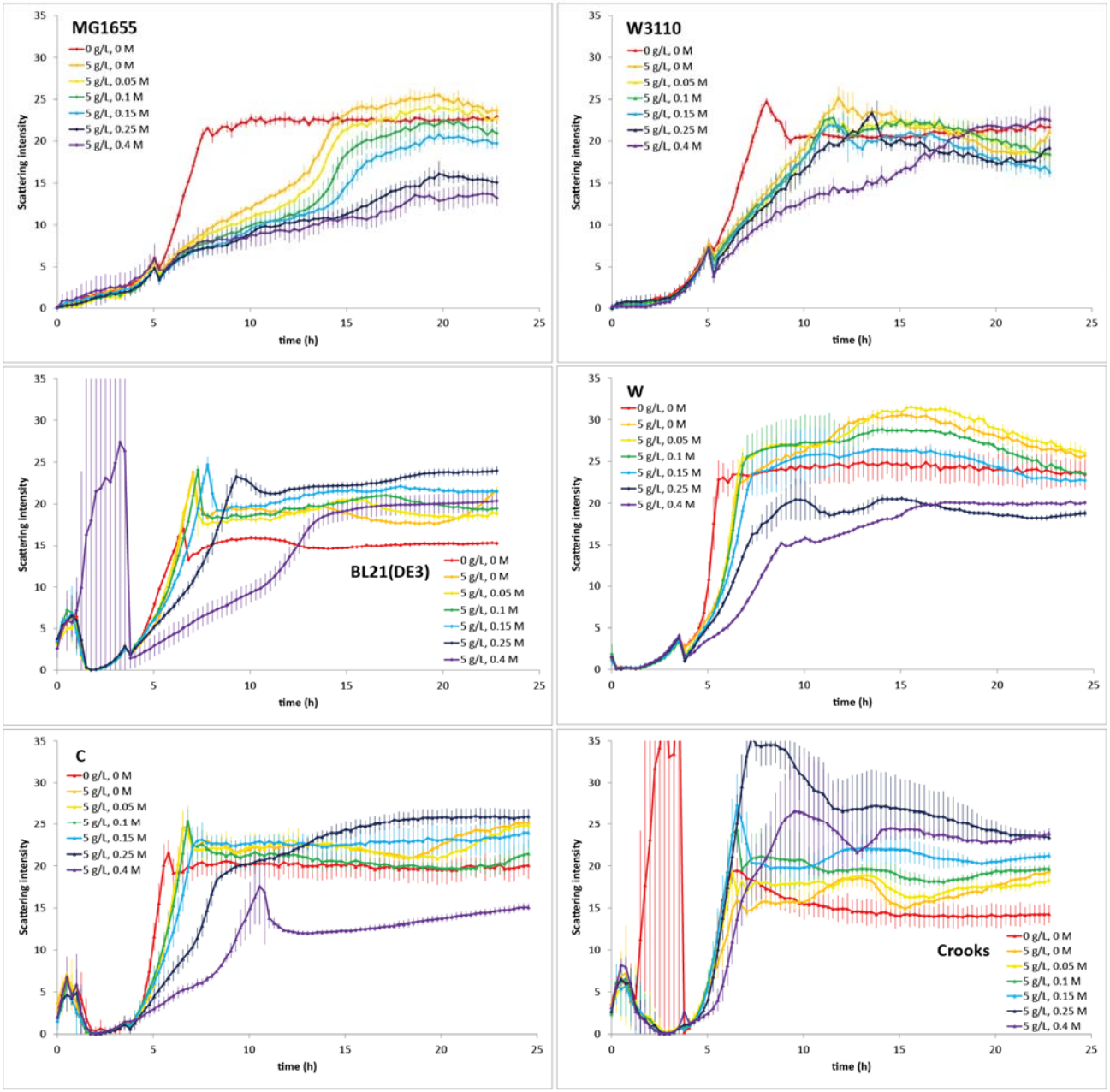


Fig. S7. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) grown with 5 g/L sodium acetate and a concentration gradient of NaCl from 0 to 0.4 M (no added sodium acetate or NaCl is shown in the red curves as a control). Sodium acetate and NaCl were added during exponential growth (5 h for MG1655 and W3110; 3.5 h for BL21(DE3), W, C, and Crooks).

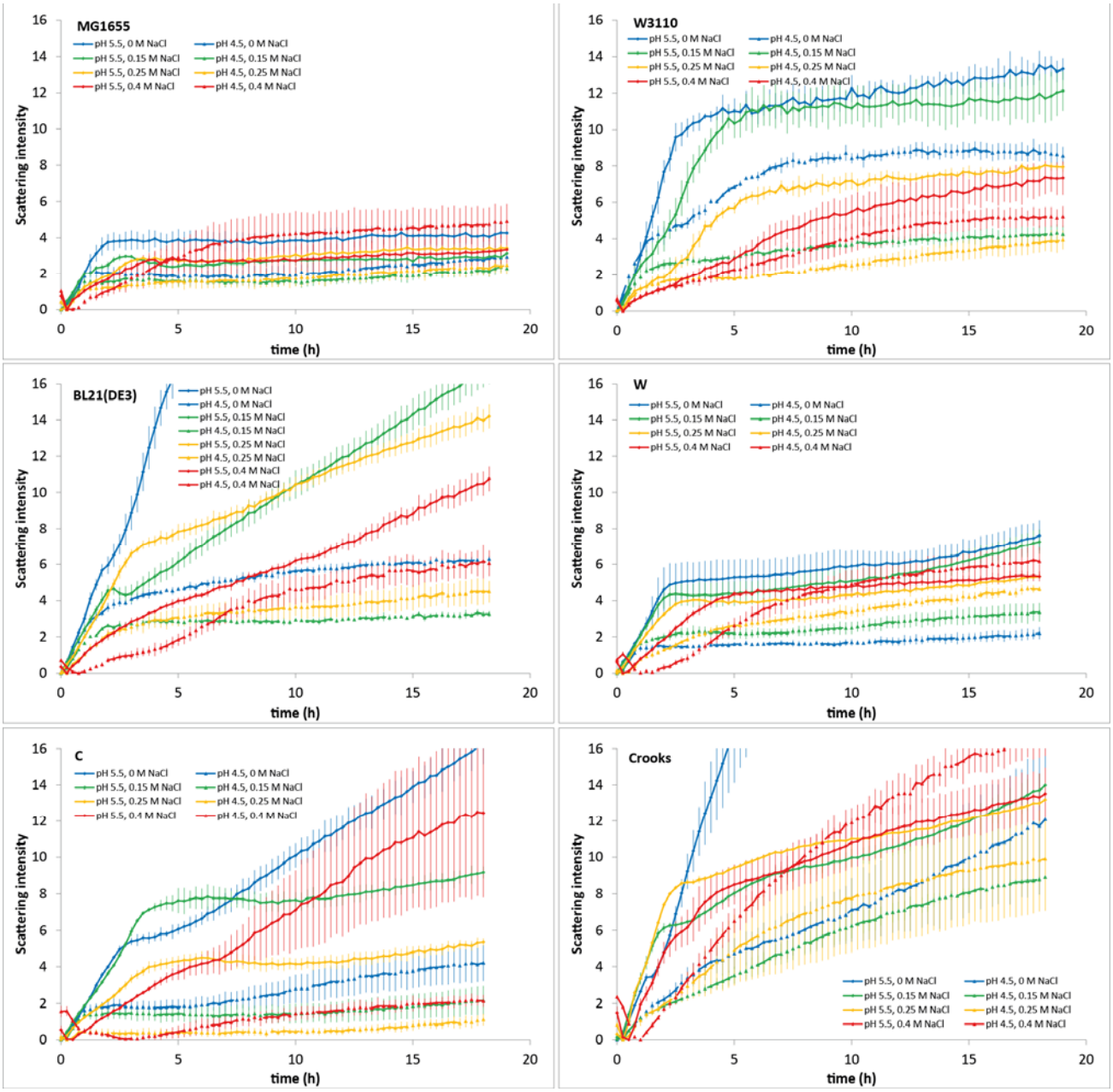


Fig. S8. Growth curves for K-12 MG1655 (top left), K-12 W3110 (top right), BL21(DE3) (middle left), W (middle right), C (bottom left), and Crooks (bottom left) grown in pH 5.5 or 4.5 and a concentration gradient of NaCl from 0 to 0.4 M. Cells were transferred to low pH media containing different NaCl concentrations during exponential growth (at the following OD_{600} values for each strains: 0.6 for K-12 MG1655, 0.5-0.6 for K-12 W3110, 0.4 for BL21(DE3), 0.4-0.5 for W, 0.4 for C, and 0.4 for Crooks). Online monitoring of growth began after transfer as described under Fig. S6.

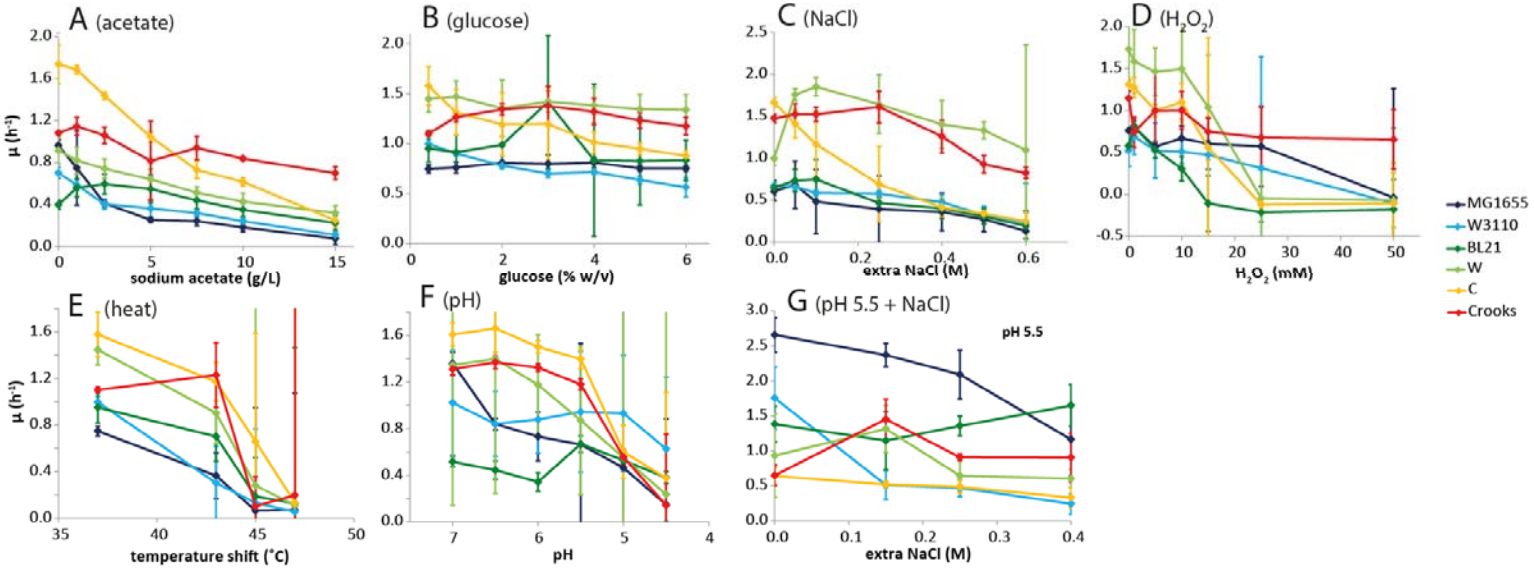


Fig. S9. Absolute growth rates (μ) for six strains of *E. coli* grown in M9 + 0.4% glucose and exposed during mid-log phase to the different stressors indicated, with the exception of high glucose concentrations, which were present from inoculation. (A) Addition of sodium acetate to final concentrations between 0 to 15 g/L. (B) Presence of varying concentrations of glucose between 0.4% to 6% w/v upon inoculation. (C) Addition of extra sodium chloride (beyond that present already in M9 medium) to final concentrations between 0 to 0.6 M. (D) Addition of hydrogen peroxide to final concentrations between 0 to 50 mM. (E) Shift to temperatures between 37°C (no shift) and 47°C. (F) Transfer of cells to M9 medium with pH values between 4.5 to 7. (G) Shift to M9 acidified to pH 5.5 and with addition of extra sodium chloride between 0 to 0.4 M. Errors bars represent propagated standard errors about the growth rate calculated for the averaged growth curve for all biological replicates.

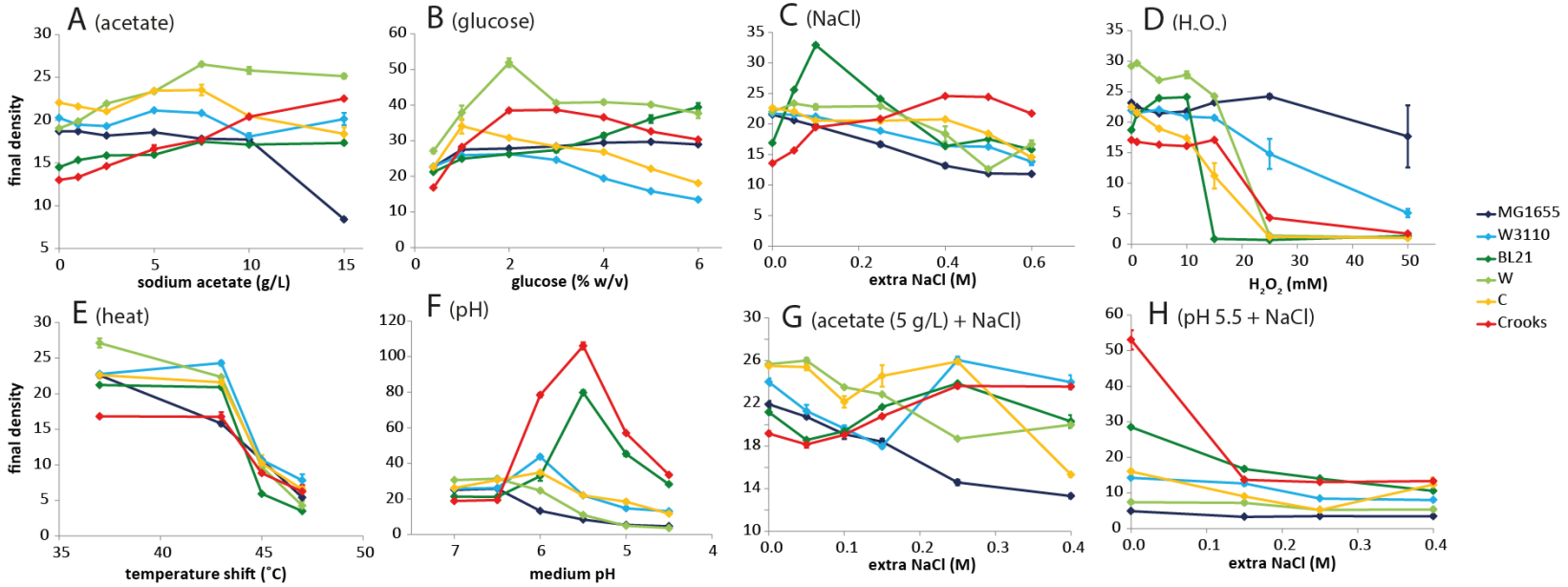


Fig. S10. Absolute final densities (arbitrary light backscatter intensity units) for six strains of *E. coli* grown in M9 + 0.4% glucose and exposed during mid-log phase to the different stressors indicated, with the exception of high glucose concentrations (B), which were present from inoculation. (A) Addition of sodium acetate to final concentrations between 0 to 15 g/L. (B) Presence of varying concentrations of glucose between 0.4% to 6% w/v upon inoculation. (C) Addition of extra sodium chloride (beyond that present already in M9 medium) to final concentrations between 0 to 0.6 M. (D) Addition of hydrogen peroxide to final concentrations between 0 to 50 mM. (E) Shift to temperatures between 37°C (no shift) and 47°C. (F) Transfer of cells to M9 medium with pH values between 4.5 to 7. (G) Addition of 5 g/L sodium acetate and extra sodium chloride between 0 to 0.4 M. (H) Shift to M9 acidified to pH 5.5 and with addition of extra sodium chloride between 0 to 0.4 M. Error bars represent standard errors about the final density calculated for the averaged growth curve for all biological replicates.

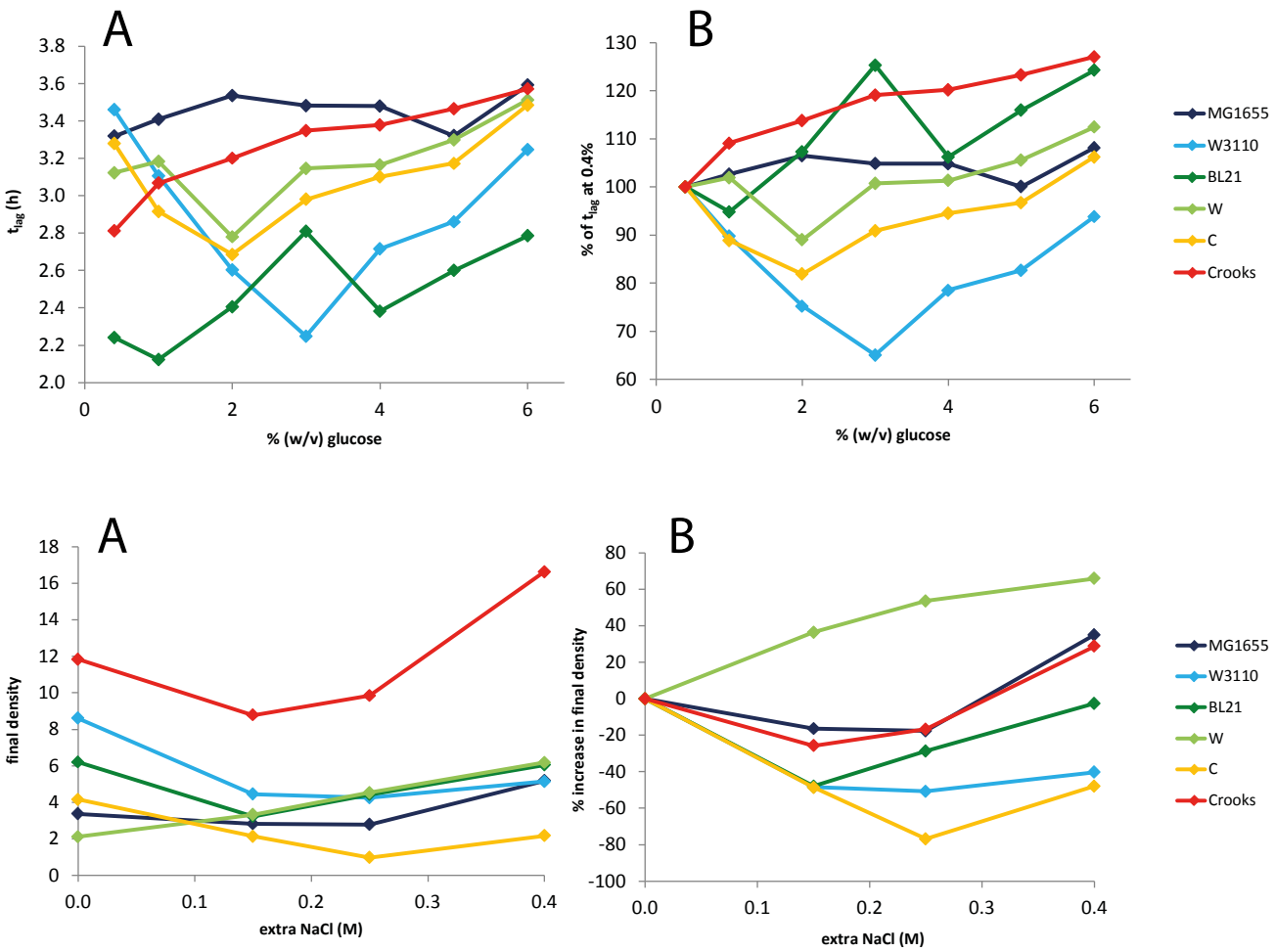


Fig. S11. Top: (A) Lag times and (B) percent of lag time for growth on 0.4% glucose for cells grown in a concentration series of glucose between 0.4% to 6%. Bottom: (A) final density and (B) percent of final density for growth at pH 4.5 with no additional NaCl, for cells transferred to media at pH 4.5 with a concentration series of NaCl between 0 to 0.4 M.

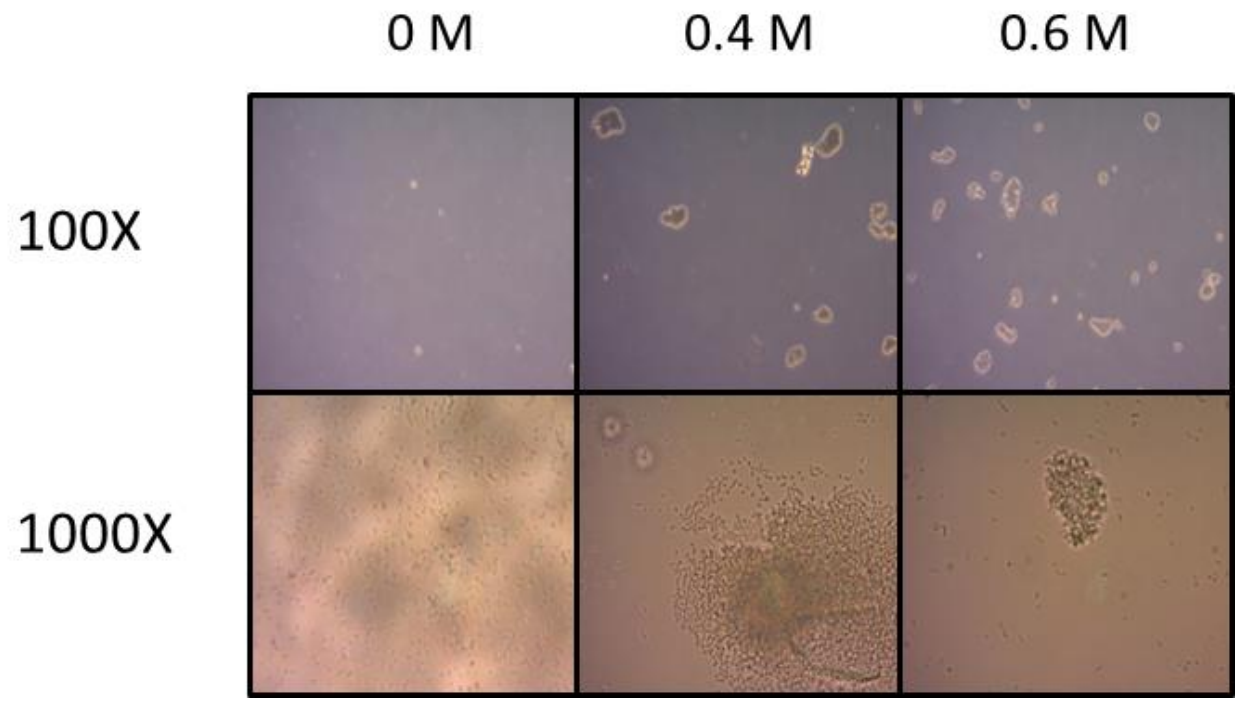


Fig. S12. *E. coli* W sampled during the early stationary phase of growth (8 hours post-inoculation) in M9 containing no additional NaCl, M9 + 0.4 M NaCl, and M9 + 0.6 M NaCl and observed directly under phase contrast microscopy at 100X and 1000X total magnification. No cell aggregants were observed under the control condition (left), with individual cells visible at 1000X magnification. Very large clumps visible at low magnification were observed with the addition of 0.4 to 0.6 M NaCl, which are the likely cause of large standard deviations in light scatter intensities in Fig. S3 for strain W.

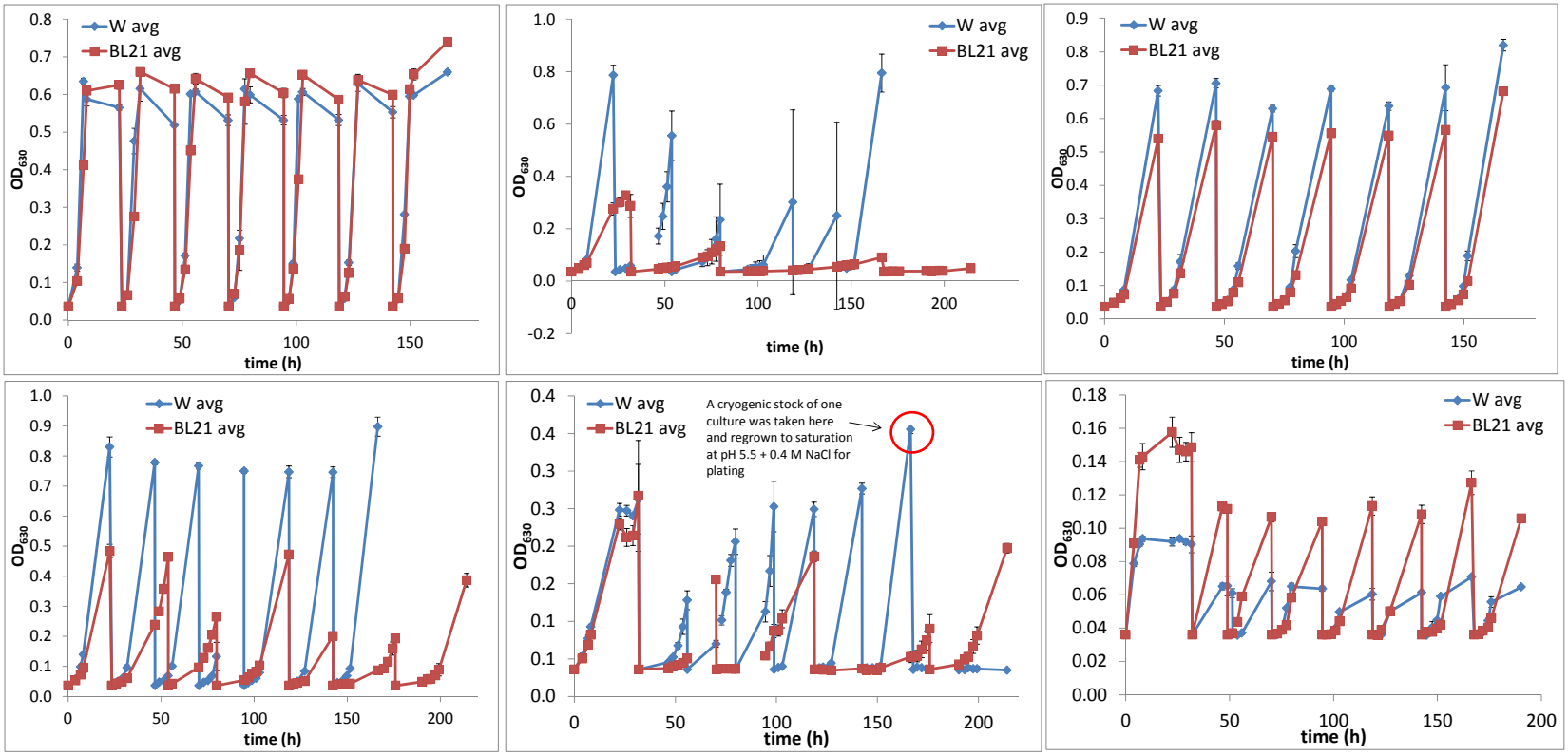


Fig. S13. Serial passaging growth profiles of Tn5 insertion libraries of W and BL21(DE3) in M9 medium (top left), M9 + 0.6 M NaCl (top middle), M9 + 15 g/L NaAc (top right), M9 + 5 g/L NaAc + 0.4 M NaCl (bottom left), M9 pH 5.5 + 0.4 M NaCl (bottom middle), and M9 pH 4.5 (top right). Optical densities were measured using 0.1 mL of cell culture in a 96 well plate at a wavelength of 630 nm on a BioTek ELx808 plate reader. Error bars represent standard deviations about the mean optical density for three replicate cultures. An error inoculating the final culture of the W library grown at pH 5.5 + 0.4 M NaCl necessitated regrowing the selected population from a cryogenic stock harvested at the time indicated. The culture reached an OD_{600} of 1.34 (spectrophotometer with 1 cm pathlength) after approximately 24 hours growth, which was nearly equal to the average OD_{600} of 1.36 measured at the circled point.

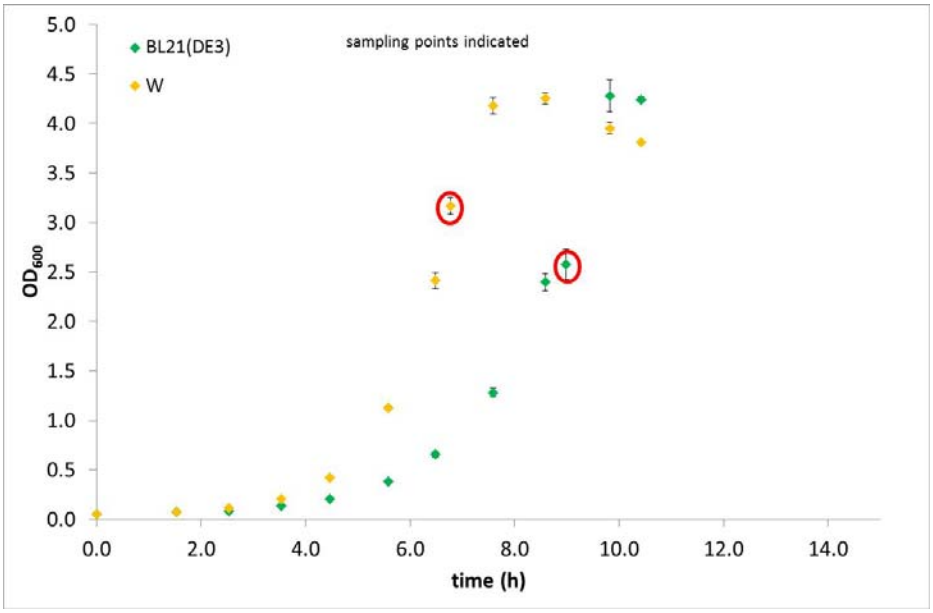
elapsed (h)	W						BL21					
	M9	0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	pH 4.5	M9	0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	pH 4.5
0	0	0	0	0	0	0	0	0	0	0	0	0
24	X	0	0	0	0	.	0	0	0	0	0	0
30		0	0	0	0	-	0	0	0	0	0	.
45		0	.	.	.	.	X	0	0	0	0	.
53.5		0	X	X	X	X		0	0	0	0	X
75.7		.						0	X	.	.	
99.3		X						.		X	X	
117								.				
144								X				
total (h)	24	99	54	54	54	54*	45	144	76	99	99	54

after serial passaging

elapsed (h)	W						BL21					
		0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl*	pH 4.5		0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	pH 4.5
0		0	0	0	0				0			
23		0	0	0	0	0			0			0
47		0	.	.	0	.			0	0	0	0
54		0	X	X	0	X			.	0	0	0
71		X			.			0	X	0	0	0
101					X			0		0	0 (X)	0
120								0		.	0	0
143								0		.	0	0
150								0		X	0	0
167								0			0	0
174								0			0	0
total (h)		71	54	54	98	31		∞	71	103	(54)	∞

Fig. S14. Estimated elapsed times to observe colony growth of Tn5 insertion libraries of *E. coli* W and BL21(DE3) on M9 plates containing high sodium chloride and/or high sodium acetate concentrations, low pH, and low pH and high sodium chloride concentrations. The top table is for cells plated directly without serial passaging, and the bottom table is for cells plated after serial passaging. '0' indicates no growth was observed at the corresponding elapsed time, '.' indicates that only very small colonies were observed, and 'X' indicates the time at which colonies were large enough to assess the size distribution (and was when colonies were selected for propagation and subsequent secondary screening). Blank entries before '0' are at times prior to when plates were spread with cells. For passaged BL21(DE3) libraries in some conditions (0.6 M NaCl, pH 5.5 + 0.4 M NaCl, and pH 4.5), no colonies were

observed to grow on plates despite growth of cells in liquid culture. Two colonies were observed for the BL21(DE3) library plated on pH 5.5 + 0.4 M NaCl plates after approximately 50 hours, however later sequencing of the Tn5 location revealed that these colonies were actually strain W.



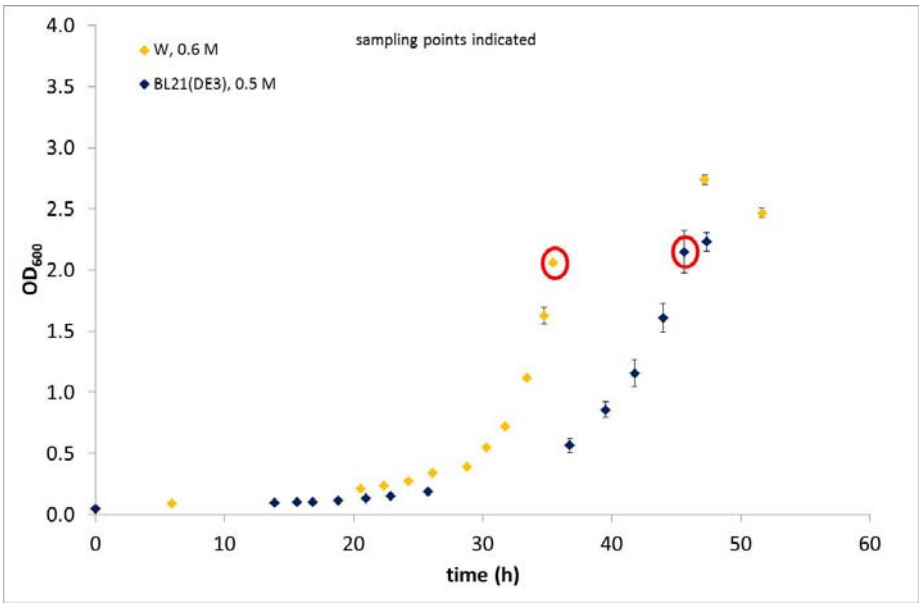


Fig. S15. Growth curves for Tn5 libraries of strains W and BL21(DE3) selected in M9 medium (top) and M9 + 0.6 M NaCl for W or 0.5 M NaCl for BL21(DE3) (bottom). Each point represents the average OD_{600} of two or three biological replicate cultures. The times when cultures were harvested for genomic DNA extraction for Tn-Seq are circled in red.

strain	% increase in final density						
	M9	0.6 M NaCl	0.4 M NaCl	15 g/L Ac	pH 5.5	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl
BL21 Δ*proV*	-0.4	**30.4***	**20.2***	1.9	7.1	10.4	50.8
W Δ*rfe*	**-29.7***	8.6	-36.8	**11.7***	**29.8***	**-34.2***	-11.8
W Δ*evgS*	1.5	-	8.0	0.2	-19.0	0.1	-12.4
W Δ*evgA*	0.1	-2.6	8.8	2.0	-15.7	**9.6***	-11.6
W Δ*typA*	**17.5***	**11.4***	-1.2	-11.3	-22.8	**22.1***	**11.8***
W Δ*rcsB*	6.4	9.0	11.4	**7.9***	8.6	-4.1	61.9
W Δ*nagC*	0.8	**-18.1***	**-13.0***	**-16.7***	**-44.4***	**-31.2***	**-48.1***
W Δ*nagA*	4.0	**-12.2***	6.3	17.2	**-27.9***	**-38.3***	**-53.3***
W Δ*yobF*	7.5	**5.9***	0.9	**-15.7***	48.3	9.6	**55.5***
W Δ*ptsP*	1.9	**19.1***	5.4	-6.1	58.8	3.2	**42.0***
W Δ*ackA*	-1.9	**9.8***	**50.5***	**-12.5***	6.1	**-29.6***	**63.3***
W Δ*ygaH*	0.9	3.7	**31.7***	3.5	13.4	0.6	6.0
W Δ*yciW*	0.6	6.1	**52.8***	-5.9	63.3	2.3	**40.6***

100
75
50
25
10
0
-10
-20
-30
-40
-50

Fig. S16: Percent increases in final cell density of single gene deletion mutants of W and BL21(DE3) under the different single and combined stresses analyzed in this study. Values are bolded if statistically significant with $P < 0.05$, and colored green if a positive value and red if a negative value. Boxed areas indicate the condition for which Tn5 insertions in the deleted genes were originally isolated.

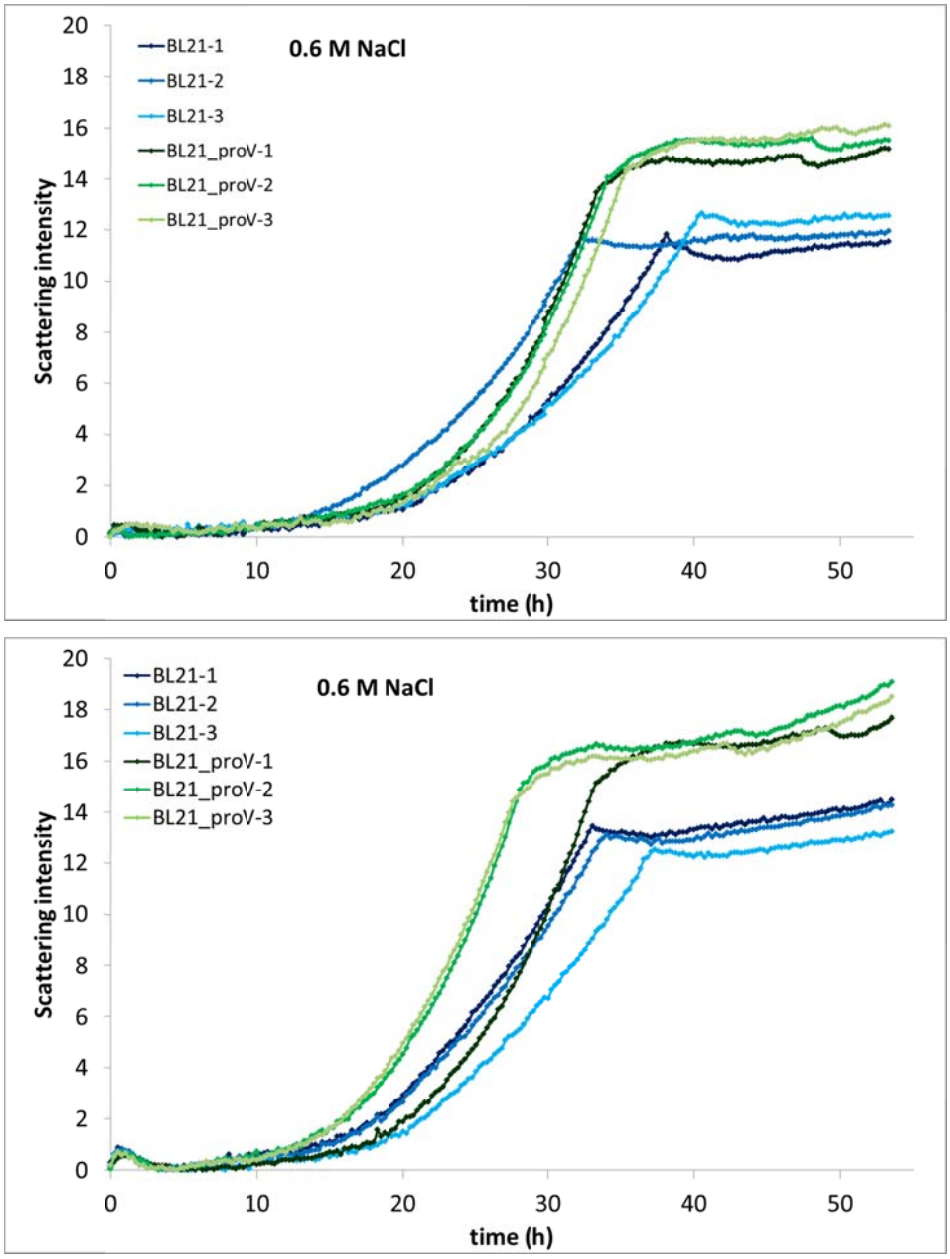


Fig. S17. Growth curves from two independent experiments with biological triplicate cultures of BL21(DE3) and BL21(DE3) Δ*proV* grown in M9 + 0.6 M NaCl in a BioLector microbioreactor system as described in Materials and Methods.

	% increase in μ over KO1, KO2						% increase in μ over W		% decrease in t_{lag} over KO1, KO2				% decrease in t_{lag} over W		% increase in final density over KO1, KO2				% increase in final density over W	
strain (1/2)	0.6 M NaCl		15 g/L Ac		5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	cond KO1 vs. W	cond KO2 vs. W	0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	cond KO1 vs. W	cond KO2 vs. W	0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	cond KO1 vs. W	cond KO2 vs. W
Δ*rfe evgA ::kan*						91	-21							25.8					38.1	
Δ*rfe typA ::kan*						178							30.9	36.7		6		108	35.9	132.2
Δ*rfe nagC ::kan*		-24						-		41			36.5	-	-17	19				-
Δ*rfe ptsP ::kan*							-13			22							-35			
Δ*rfe yobF ::kan*			9	10					-9	25				23.3		-8	17			
Δ*rfe nagA ::kan*		-13						-13.8	-15	21			17.0	-	-17	12				-
Δ*rfe ackA ::kan*		-15						-16.7	-34	19			16.0	22.7	-18				6.9	-33.6
Δ*rfe ygaH ::kan*									-59			23				30		-26	15.7	
Δ*rfe yciW ::kan*								-11.3		25			25.1			13		-56	11.2	
Δ*evgA typA ::kan*								-				10 12	34.7	-						-
Δ*evgA nagC ::kan*						-46	61		-15			36	18.9	-11.4	-26			-65	-47.1	-21.6
Δ*evgA ptsP ::kan*																	-37	60		
Δ*evgA yobF ::kan*				23		36	65	190.9				8 39		5.4		-11		40 125	122.5	-11.5
Δ*evgA nagA ::kan*	-12	11							-24	-23		-30			-21	-7		-58	-46.8	-23.2
Δ*evgA ackA ::kan*					-5		69	106.7				25	27.8				-34	59 79	191.8	-32.8
Δ*evgA ygaH ::kan*						-34						-18 16		-				-39		-
Δ*evgA yciW ::kan*						-17	39	87.8	-			32	44.4	-				65 26	204.0	-
Δ*typA nagC ::kan*		11				-45	116		-14			16 41	18.1		-24	18		-66 41	-51.3	-9.0
Δ*typA yobF ::kan*								104.3			-22	8 42	34.8					92	89.8	
Δ*typA nagA ::kan*	-13	-12					38	58.4	-12.9	-18	-13	-60	-41.6	-18.4	-21			-61	-50.4	-12.4
Δ*typA ackA ::kan*							54	87.9	15.1			-12 29	32.0	11.2			-27	34 70	177.8	-14.0
Δ*typA ygaH ::kan*						-61	-48	-38.2	-			-59	-27.6	-				-38 -32		-
Δ*typA yciW ::kan*							66	125.3	-			22	36.7	-				21	152.4	-
Δ*nagC ptsP ::kan*		10			28	-35			-21.0			20				-19	60	-32	-11.4	-24.2
Δ*nagC yobF ::kan*				-21					-26.7		10 -24					-23				-13.8
Δ*nagC ackA ::kan*	-36	-25			23	-35		-39.4	-24.1			19			-60	-70	27	-16	-67.2	-39.9
Δ*nagC ygaH ::kan*	-19	-22					-45	-65	-24.7	-66.4	-11 -24	-33 -76	-23.7	-67.0	-22	-43		-28 -68	-40.8	-61.7
Δ*nagC yciW ::kan*								-54	-39.4	14			4.7		13	-19		21 -63	-14.5	-35.4
Δ*ptsP typA ::kan*						56						29		26.7				50 8	23.8	
Δ*ptsP yobF ::kan*				10	14	14		30.3			15		7.4	27.0		-9				
Δ*ptsP nagA ::kan*	13				-33	23		-18.0		-27 -24		-19 -17	-11.0		-24		-30	23	-26.8	-18.1
Δ*ptsP ackA ::kan*						14		21.7	-					-			-27		-28.3	-
Δ*ptsP ygaH ::kan*							-51	-50	-50.6			-16 -32	-7.6					-33		
Δ*ptsP yciW ::kan*					14	10			30.6				11.2					17		64.6
Δ*yobF nagA ::kan*	16	32	-31							-18					-8	11				-9.0
Δ*yobF ackA ::kan*			10	20				52.9								-9	-30	13		-27.2
Δ*yobF ygaH ::kan*				59			-33	50.3	-28.7					-19.4				53		43.6
Δ*yobF yciW ::kan*													14.9			-9 -17			-21.7	67.7
Δ*nagA ackA ::kan*	-29	-18			21	-20		-28.4	-12.4	-45		16	-26.9		-17	-29		-11	-28.8	-42.5
Δ*nagA ygaH ::kan*	-14	-10					-30	-32	-12.7	-35.3	-28	-34 -22		-42.1	-15	-24		-44	-27.3	-51.0
Δ*nagA yciW ::kan*		11						-38		-25 -18		-17		-21.3	-3	-19		-51	-17.1	-36.2
Δ*ackA ygaH ::kan*					-52	-44	-57	-60	-43.6	-57.1		-4	-15	-11.9 -34.5				-51 -57	-53.2	-47.5
Δ*ackA yciW ::kan*											9	5 -8	10.9	-9.5			16	-30 48 13	-26.2	79.8
Δ*ygaH yciW ::kan*						19	-19		-					-				46	28.1	-

Fig. S18. Percent increases in growth rate, percent decreases in lag time, and percent increases in final cell density for strains containing combinations of deletions in two genes under the stress conditions indicated. For a given strain name ΔA B::kan, the first column for each condition represents the percent change relative to strain ΔA, while the second column represents the percent change relative to strain ΔB. Percent changes compared to the background strain W are also listed for condition 1 and condition 2, where condition 1 is the condition under which the strain containing Tn5 insertion(s) in gene A was originally isolated, and condition 2 is the condition under which the strain containing Tn5 insertions in gene B was originally isolated. Only statistically significant values with $P < 0.05$ are shown, with color shaded according to the value using a green-yellow-red scale (dark green = 50%, yellow = 0%, dark red = -50%). Strains with positive values in 2 columns for any one condition exhibit positive epistasis and are outlined in blue. Strains are also highlighted blue if they have increased percentages over strain W in two conditions.

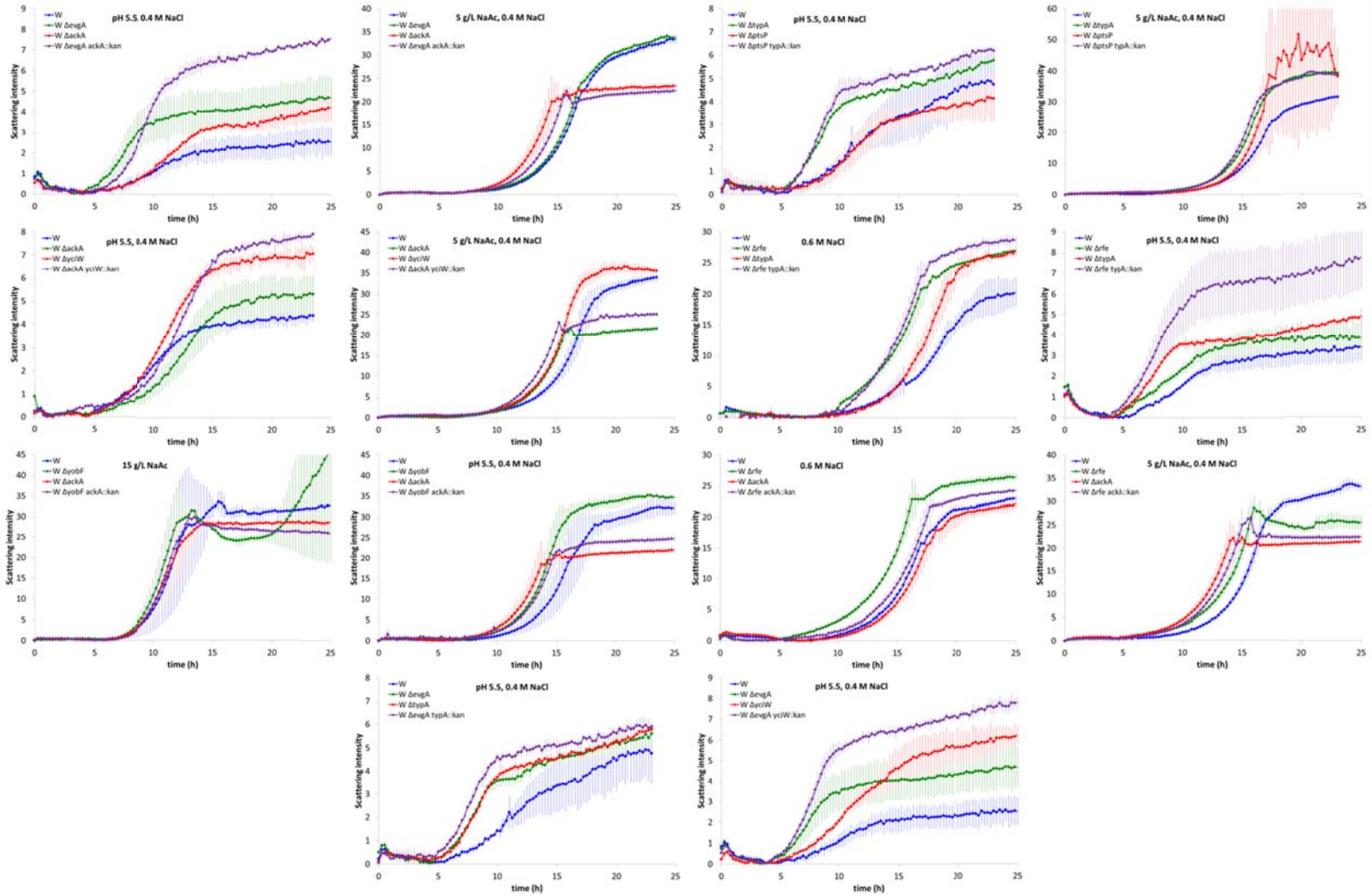


Fig. S19. Growth curves for selected double deletion strains for conditions indicated. The strains shown are W Δ*evgA ackA*::*kan* and W Δ*ptsP typA*::*kan* (top row), W Δ*ackA yciW*::*kan* and W Δ*rfe typA*::*kan* (second row), W Δ*yobF ackA*::*kan* and W Δ*rfe ackA*::*kan* (third row), and W Δ*evgA typA*::*kan* and W Δ*evgA yciW*::*kan* (bottom row) under one or two indicated conditions.

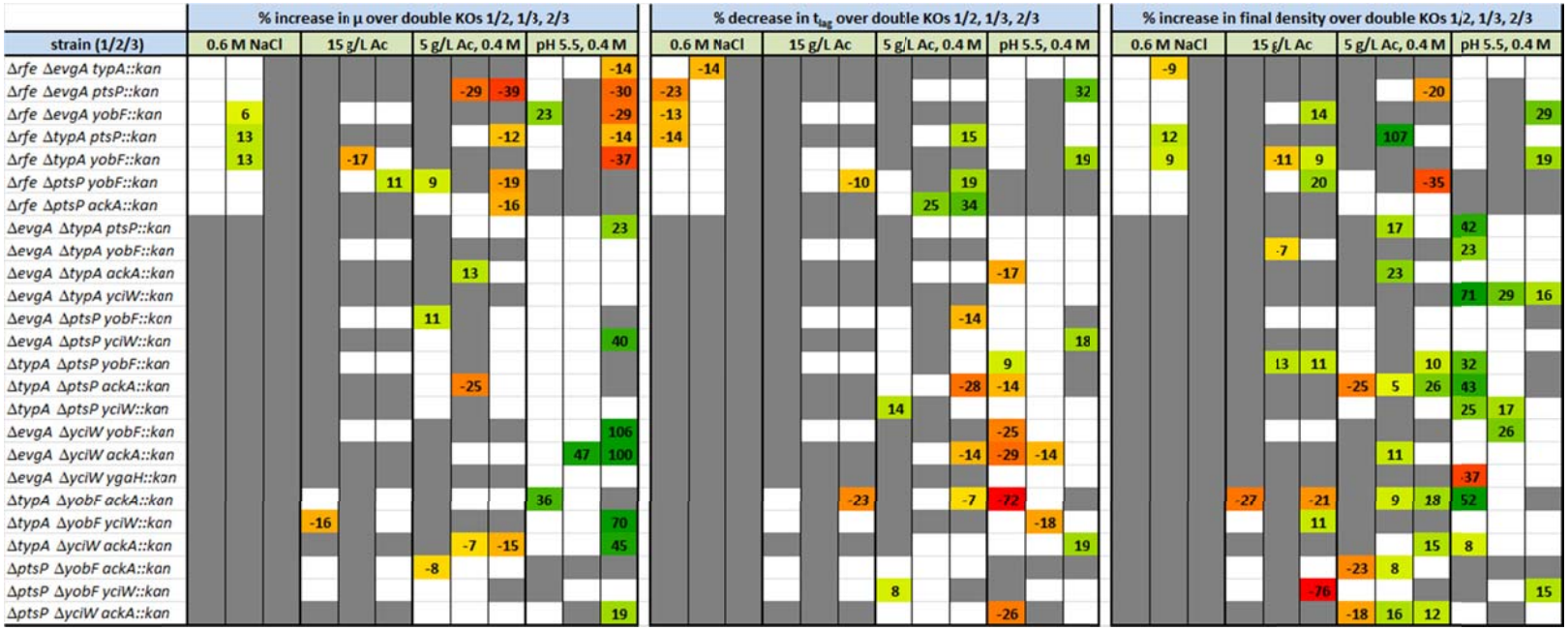


Fig. S20. Percent increase in growth rate for triple knockouts (for W ΔA ΔB C::kan, the first column for each condition is compared to W ΔA ΔB, the second column is compared to W ΔA ΔC, and the third column is compared to W ΔB ΔC). Only statistically significant values with $P < 0.05$ for comparison of the mean values are shown. Gray cells indicate that the condition was not tested. The color is shaded according to the value using a green-yellow-red scale (dark green = 50%, yellow = 0%, dark red = -50%).

strain	percent increase in μ over W				percent decrease in t_{lag} over W				percent increase in final density over W			
	0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl	0.6 M NaCl	15 g/L Ac	5 g/L Ac, 0.4 M NaCl	pH 5.5, 0.4 M NaCl
Δrfe ΔevgA typA::kan	**-16**			**25**	**5**			**37**	**14**			**56**
Δrfe ΔevgA ptsP::kan	**-14**		**-27**	**45**	**28**		-5	**56**	**17**		**-20**	**70**
Δrfe ΔevgA yobF::kan	**-16**	-1		**64**	**25**	9		**35**	**21**	-2		**145**
Δrfe ΔtypA ptsP::kan	-1		**10**	**66**	**14**		**17**	**38**	**32**		**9**	**16**
Δrfe ΔtypA yobF::kan	**-10**	**-18**		**49**	**21**	3		**31**	**31**	**-14**		**79**
Δrfe ΔptsP yobF::kan	**-19**	7	**13**		**15**	1	**33**		**19**	-4	**-20**	
Δrfe ΔptsP ackA::kan	**-16**		8		**26**		**52**		**20**		-15	
ΔevgA ΔtypA ptsP::kan			**27**	**56**			4	27			**17**	**121**
ΔevgA ΔtypA yobF::kan		**-19**		**157**		8		**16**		**-20**		**64**
ΔevgA ΔtypA ackA::kan			**23**	**87**			1	**14**			**-16**	**78**
ΔevgA ΔtypA yciW::kan				**99**				**44**				**134**
ΔevgA ΔptsP yobF::kan		**21**	**45**	**81**		**25**	4	**36**		**51**	**23**	**76**
ΔevgA ΔptsP yciW::kan			**56**	**119**			**13**	**30**			3	**70**
ΔtypA ΔptsP yobF::kan		2	**33**	**92**		-1	2	**27**		**-11**	**16**	**105**
ΔtypA ΔptsP ackA::kan			**29**	**80**			3	8			**-12**	**122**
ΔtypA ΔptsP yciW::kan			**24**	**70**			**28**	**49**			**15**	**136**
ΔevgA ΔyciW yobF::kan		**9**		**105**		**35**		**35**		**-9**		**112**
ΔevgA ΔyciW ackA::kan			**16**	**127**			**18**	**14**			**-26**	**54**
ΔevgA ΔyciW ygaH::kan				**57**				**19**				**5**
ΔtypA ΔyobF ackA::kan		**13**	**21**	**56**		**12**	**19**	**17**		**-36**	**-11**	**91**
ΔtypA ΔyobF yciW::kan		**-10**		**70**		**34**		**41**		**-14**		**114**
ΔtypA ΔyciW ackA::kan			**12**	**64**			**26**	**19**			**-14**	**72**
ΔptsP ΔyobF ackA::kan		**42**	**22**			**34**	**19**			**9**	**-21**	
ΔptsP ΔyobF yciW::kan		**41**	**41**	**37**		**26**	**17**	**13**		**-15**	**6**	**68**
ΔptsP ΔyciW ackA::kan			**25**	**35**			**21**	**-10**			**-15**	**60**

Fig. S21. Percent increases in growth rate (left), lag time (middle), and final density (right) for triple knockout strains of W compared to the averaged values from multiple independent experiments for strain W. Bolded values are statistically significant with $P < 0.05$. Gray cells indicate that a condition was not tested. The color is shaded according to the value using a green-yellow-red scale (dark green = 50%, yellow = 0%, dark red = -50%).

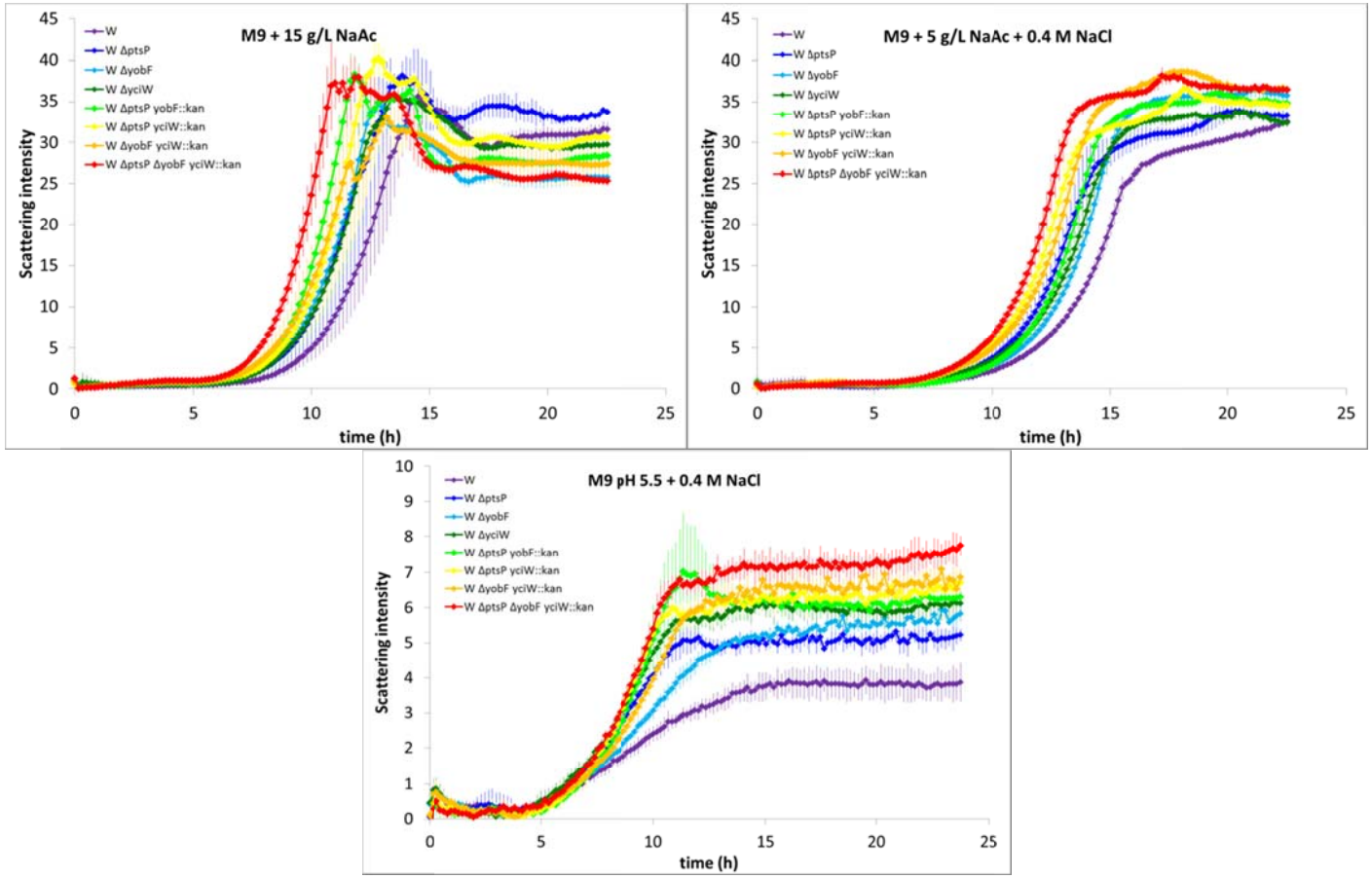


Fig. S22. Averaged growth curves for three biological replicates of strain W Δ*ptsP* Δ*yobF yciW::kan* and all comprising single and double knockouts under three different conditions (M9 + 15 g/L NaAc, M9 + 5 g/L NaAc + 0.4 M NaCl, and M9 pH 5.5 + 0.4 M NaCl). W was also grown in the same experiment as a control. The error bars represent standard deviations about the mean of the scattering intensity at each time measured for the three biological replicates.

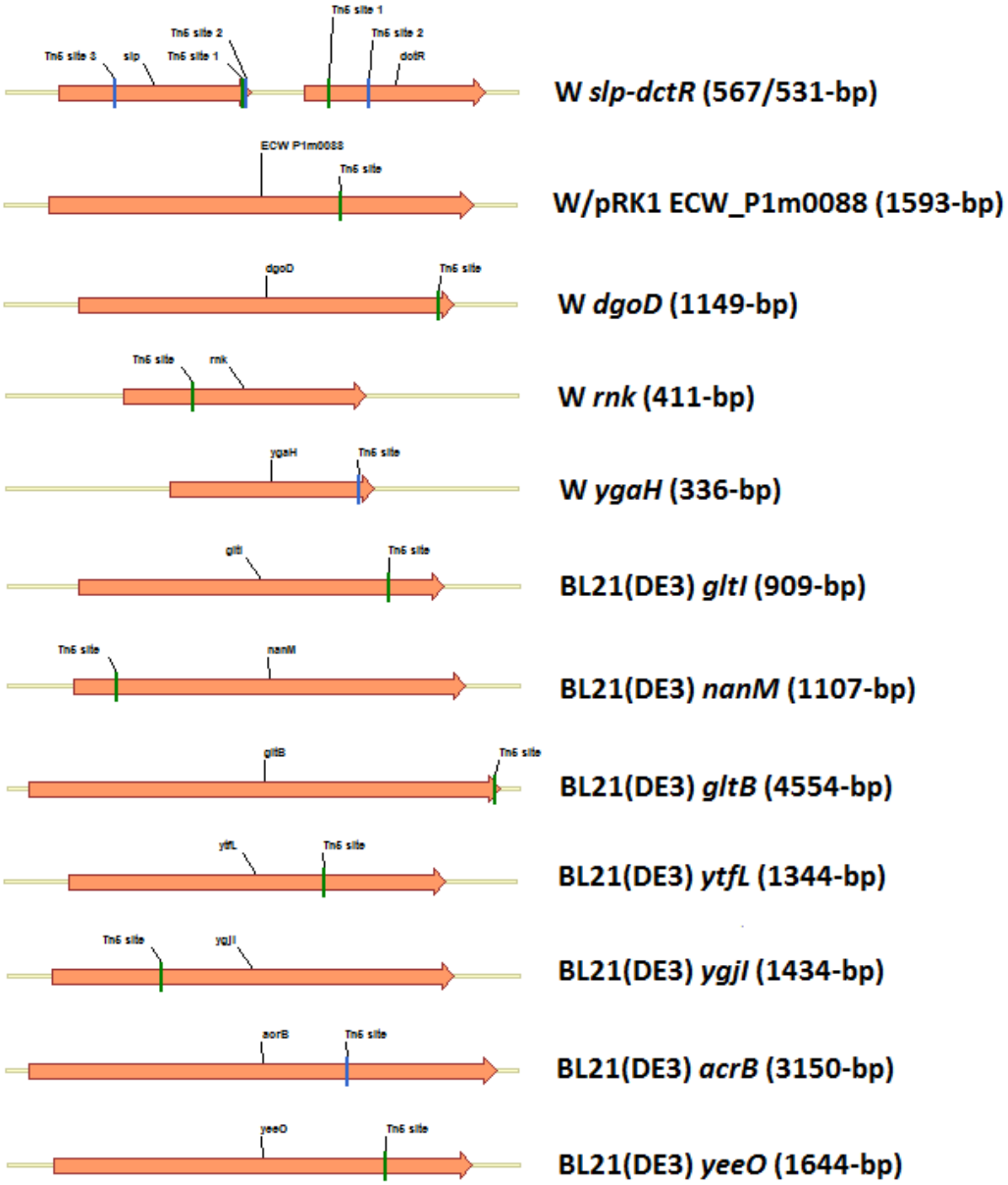


Fig. S23. Gene insertion locations in strains exhibiting improved growth properties under different stress conditions (see main text), but for which strains with clean deletions of the gene did not display improved growth by either an increased growth rate or reduced lag time.

Supplementary References

1. **Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H.** 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Sys. Biol.* **2:**2006.0008.