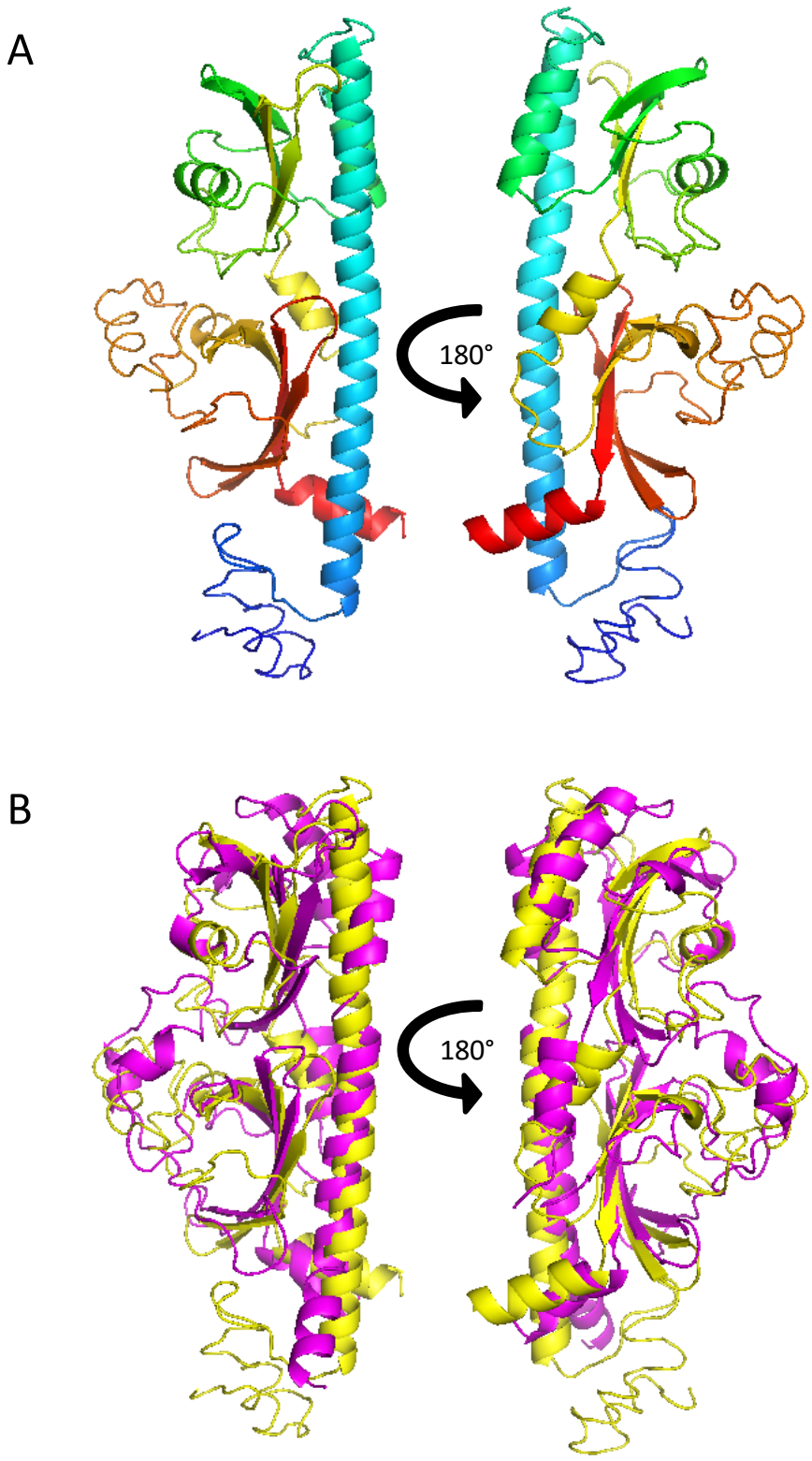

Figure S1. I-TASSER model of $RsbU_{1-315}$ shares structural similarity to periplasmic domain $RsbU_{45-313}$ crystal structure. A) I-TASSER protein structure model with residues 1 through 315 of *C. trachomatis* RsbU. **B)** Structure overlay of $RsbU_{45-313}$ crystal structure (magenta) and I-TASSER model (yellow). Structure comparison has Z-score of 14.4 and a RMSD of 4.0 Å.

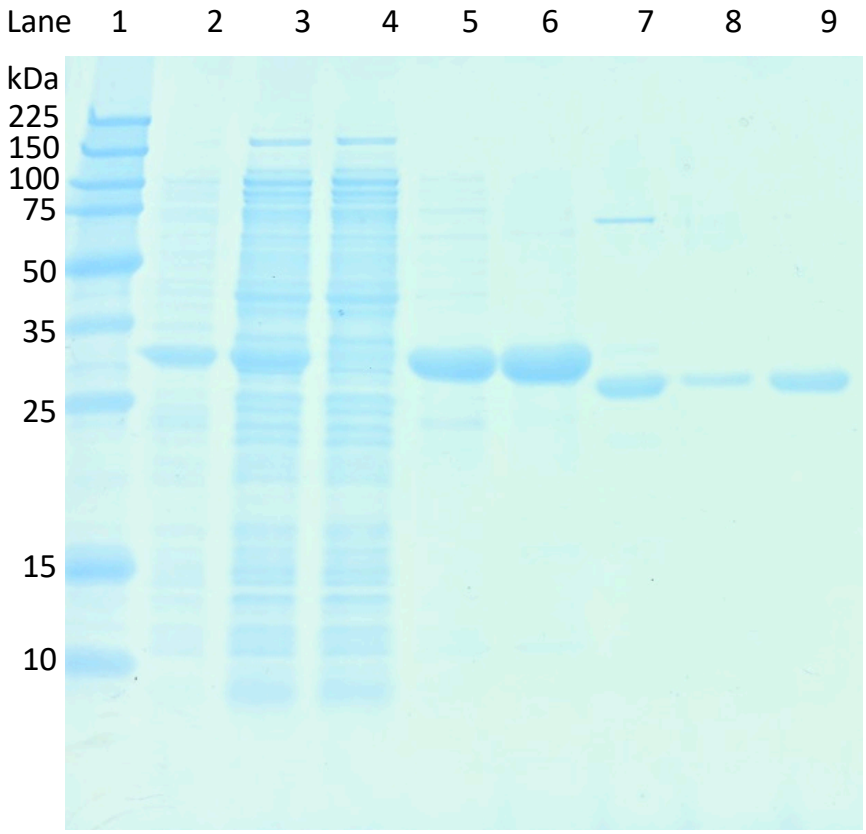

Figure S2. SDS-PAGE gel showing purification of $RsbU_{45-313}$. Lane 1, protein marker; lane 2, lysate supernatant; lane 3, lysate pellet; lane 4, IMAC flow-through; lane 5, IMAC elution; lane 6, post-buffer exchange; lane 7, post-TEV protease treatment; Lane 8, post-reverse nickel column; lane 9, SEC column peak fraction.

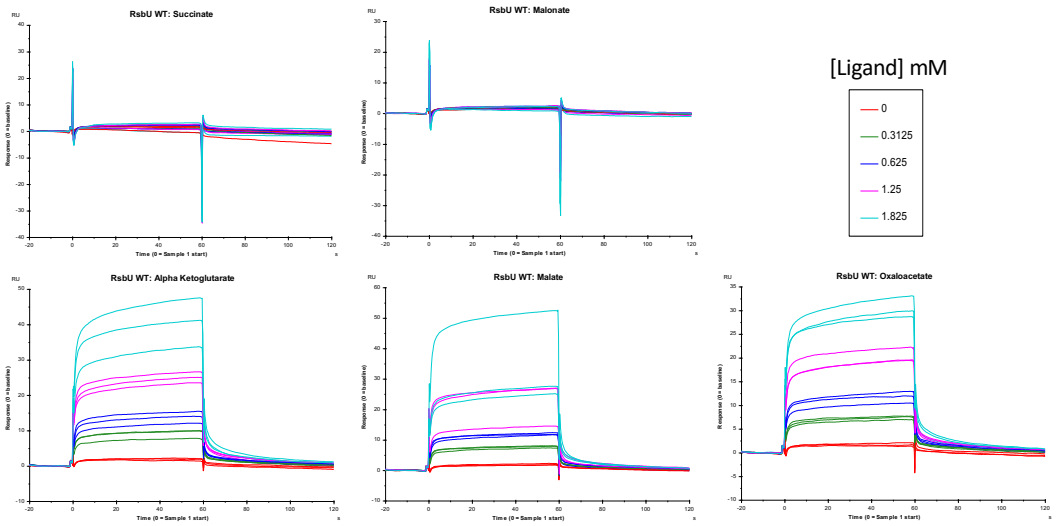

Figure S3. SPR dose-dependent binding curves. Alpha-ketoglutarate, malate, and oxaloacetate show dose-dependent binding to $RsbU_{45\text{-}313}$, while succinate and malonate, the ligands found to bind to DctB, do not appear to be binding by SPR.

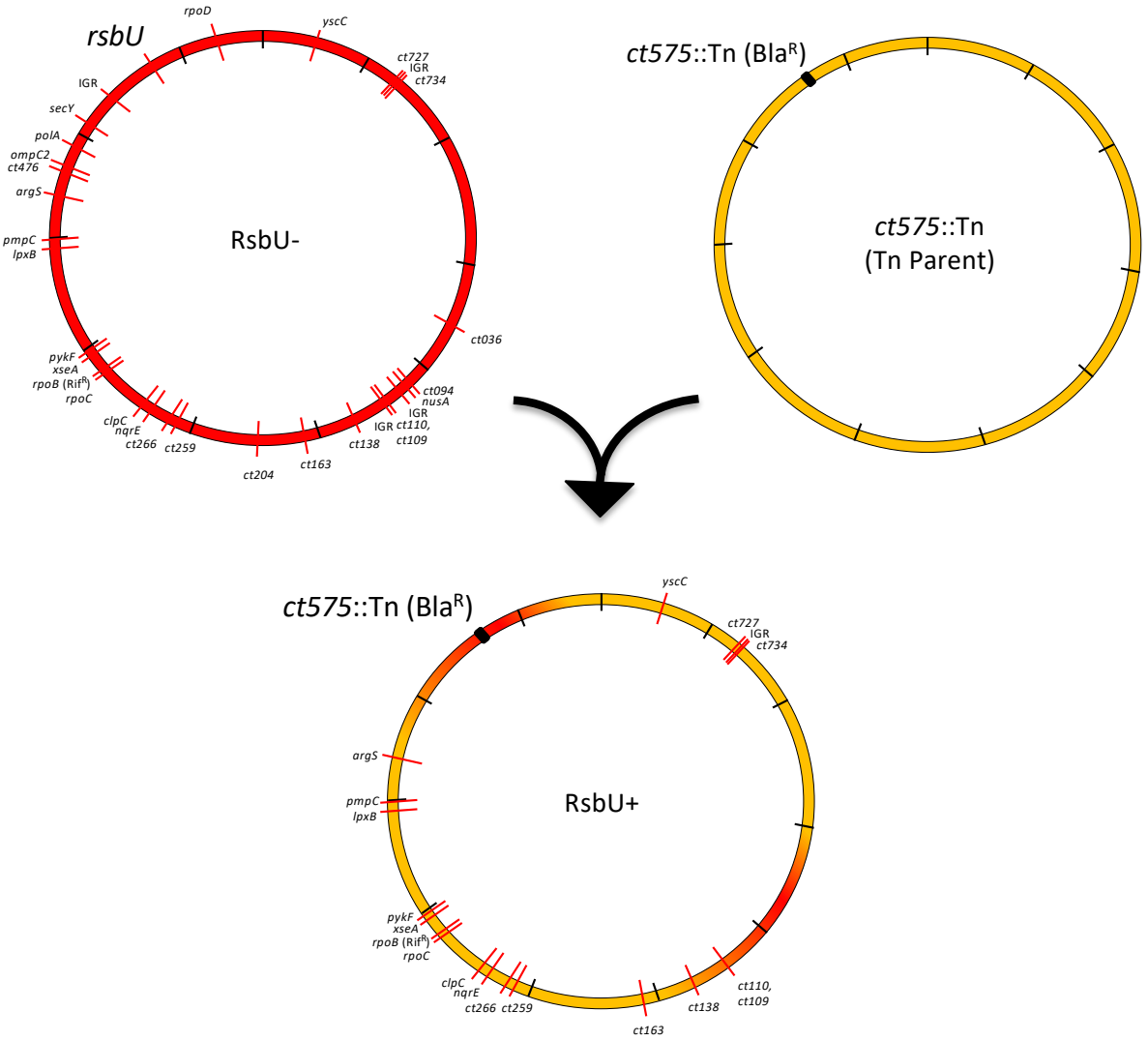

Figure S4. Chromosome schematic of cross between RsbU* EMS strain and *ct575*::Tn strain to create the complemented RsbU+ strain that retains the majority of the other SNPs induced by EMS mutagenesis. The *ct575*::Tn strain contains a beta-lactamase resistance gene in the transposon, while the RsbU* has a SNP in the *rpoB* gene that incurs rifampicin resistance. Dual selection with ampicillin and rifampicin was utilized to select for recombinants that retained the RsbU* strain backbone but a wild-type version of the *rsbU* gene. The red region on the RsbU+ chromosome represents the region of recombination between the genomes confirmed by PCR of the SNPs present in the RsbU* strain. In addition to the restoration of the *rsbU* gene, the RsbU+ strain restores three other SNPs close to the position of the transposon: two in coding regions for *secY* and *polA;* and one in an intergenic region (IGR).

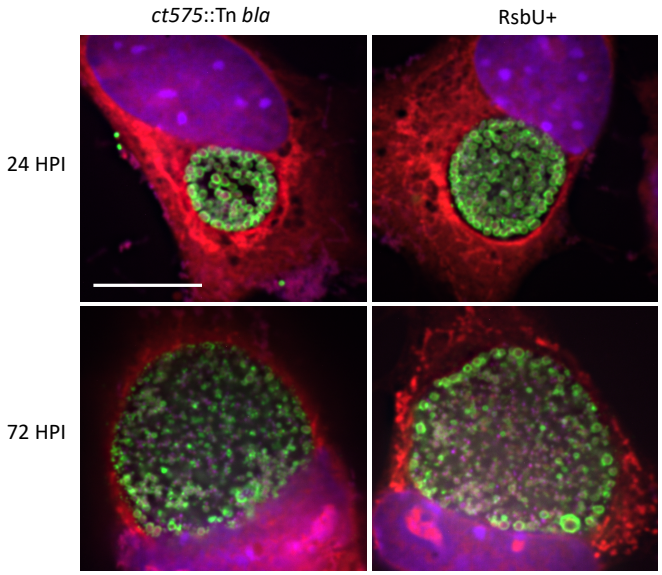

Figure S5. Immunofluorescent microscopy of *C. trachomatis ct575*::Tn parent strain and RsbU+ complemented strain. At 24 and 72 hours post-infection, the RsbU+ complemented strain does not appear to be phenotypically different from the Tn parent strain.

Table S1. Top structural homologs of $RsbU_{1-315}$ I-TASSER model.

Protein Name : Ligand Organism	Z-score	Protein Description[a]	Best Match PDB code
PctB:L-Arginine *Pseudomonas*	21.0	Methyl-accepting chemotaxis protein	5lt9[b]
Tlp3 *Campylobacter*	20.0	Methyl-accepting chemotaxis protein	4wy9[b]
mmHK1S-Z3 *Methanosarcina*	19.6	Histidine kinase sensor protein	3lib
Mlp24:Asparagine *Vibrio*	19.4	Methyl-accepting chemotaxis protein	6ior
McpX: Proline betaine *Sinorhizobium*	18.9	Methyl-accepting chemotaxis protein	6d8v
TlpQ:Histamine *Pseudomonas*	18.7	Methyl-accepting chemotaxis protein	6fu4
soHK1S-Z6 *Psuedomonas*	18.2	Histidine kinase	3lic
rpHK1S-Z16 *Shewanella*	17.5	Histidine kinase sensor protein	3lif[b]
TlpA *Helicobacter*	17.5	Methyl-accepting chemotaxis protein	6e0a
Dsm5692 *Desulfohalbium*	17.0	Histidine kinase sensor protein	5ere[b]
DctB:malonic acid *Sinorhizobium*	16.7	Histidine kinase sensor protein	2zbb[b]
Tlp3:Isoleucine *Campylobacter*	16.7	Methyl-accepting chemotaxis protein	4xmq[b]
LEP15460 *Leptospira*	16.6	Methyl-accepting chemotaxis protein	6pzj
DctB:Succinate *Vibrio*	16.1	Histidine kinase sensor protein	3by9[b]
vpHK1S-Z8:Sulfate *Vibrio*	16.0	Histidine kinase sensor protein	3lid[b]

[a] All structural homologs are periplasmic-localized, ligand-binding regions (chemosensors).

[b] Protein structures also similar to RsbU crystal structure following DALI search.

Table S2. Non-redundant structural matches for $RsbU_{45-313}$ from *C. trachomatis*.

Protein Name : Ligand Organism	Z-score	RMSD (Å)	% ID to $RsbU_{45-313}$	% ID to binding pocket (residues 111-189)	Best Match PDB code
DctB:succinate *V. cholerae*	17.8	3.2	13	6	3by9
DctB:malonate/succinate *S. meliloti*	16.5	3.7	12	0	3e4p
PctA:L-Met *P. aeruginosa*	15.1	3.71	12	0	5ltx
vpHK1S-Z8:acetate *V. parahaemolyticus*	14.5	3.8	9	0	2pj7
PctB:L-Gln *P. aeruginosa*	14.3	3.5	11	0	5lto
Tlp3:isoleucine *C. jejuni*	13.8	4	15	16	4xmq
Mlp37:alanine *V. cholera*	13.8	4.4	11	0	5avf
PctC:GABA *P. aeruginosa*	13.8	3.5	12	0	5ltv
Histidine kinase *R. palustris*	13.5	4.1	9	8	3lif
McpN *V. cholerae*	13.5	4.1	11	0	3c8c
KinD:pyruvate *B. subtilis*	13.2	3.8	12	0	4jgo
Dret_0059:cysteine *D. retbaense*	13	4.9	11	0	5ere
Tlp1 *C. jejuni*	12.8	4.4	13	0	4wy9
AHK4:isoentyladenine *A. thaliana*	11.5	4	11	5	3t4t

Table S3. Potential ligands tested for $RsbU_{45-313}$ binding.

Ligand	Compound	Mass in Daltons
Succinate	Sodium Succinate Dibasic Hexahydrate	116
Malonate	Sodium Malonate Dibasic Monohydrate	102
Glutamate	L-Glutamic Acid monosodium salt monohydrate	147
Alpha-ketoglutarate (2-oxoglutarate)	Alpha-Ketoglutaric Acid	146
Fumarate	Fumaric Acid	114
Oxaloacetate	Oxaloacetic Acid	130
Malate	D-Malic Acid	132
2- phosphoglycerate	L-2-Phosphoglyceric Acid Disodium Salt Hydrate	186
Glucose	D-(+) Glucose	180
Pyruvate	Sodium Pyruvate	87
Phosphoenolpyruvate	Phospho(enol)pyruvic Acid Monopotassium Salt	165
ATP	Adenosine 5'-triphosphate disodium salt hydrate	507
Mannitol	Mannitol	182
Lysine	L-Lysine	146
Isoleucine	L-isoleucine	131
Succinyl-CoA	Succinyl coenzyme A sodium salt	867
Pyridoxine	Pyridoxine, Vitamin B6	169
Glucosamine	Glucosamine hydrochloride	179
D-Mannose	D-(+)-Mannose	180
5-hydroxy-L-lysine	DL-5-hydroxylysine hydrochloride	162
D-talose	D-(+)-talose	180
D-galactose	D-(+)-galactose	180
D-gulose	D-(+)-gulose[1]	180
D-idose	D-idose[1]	180
7-8-dihydroneopterin	7-8-dihydroneopterin[2]	255
D-allose	D-allose[1]	180

All compounds were acquired from Sigma Aldrich unless otherwise specified.

[1] Carbosynth (United Kingdom)

[2] Toronto Research Chemicals (Canada)

Table S4. Average ΔTm of $RsbU_{45-313}$ with potential ligands.

	Concentration (mM)							
Ligand	1.25		2.5		5		10	
Alpha-ketoglutarate	Average ΔTm	SD	Average ΔTm	SD	Average ΔTm	SD	Average ΔTm	SD
Biological Replicate 1	-0.16	0.03	-0.17	0.07	0.15	0.17	1.64*	0.33
Biological Replicate 2	-0.05	0.11	0.11	0.42	1.42*	0.38	3.16*	0.10
Malate								
Biological Replicate 1	0.02	0.01	0.21	0.06	0.52*	0.07	2.35*	0.16
Biological Replicate 2	0.33	0.20	0.28	0.06	1.45*	0.87	1.64*	0.11
Oxaloacetate								
Biological Replicate 1	-0.05	0.05	0.05	0.07	0.27	0.07	1.75*	0.27
Biological Replicate 2	-0.02	0.03	0.11	0.51	-1.33	0.25	0.29	0.20
Malonate								
Biological Replicate 1	-0.09	0.03	-0.24	0.02	-0.27	0.04	-0.59	0.09
Biological Replicate 2	-1.27	1.19	0.04	0.25	0.10	0.49	-0.83	0.14
Succinate								
Biological Replicate 1	0.03	0.14	-0.25	0.04	-0.30	0.03	-0.57	0.01
Biological Replicate 2	-0.05	0.34	-0.25	0.21	-1.50	0.78	-0.20	0.34

Values in green are positive temperature shifts. Technical triplicates were performed for each biological replicate.
* p-value < 0.05 by two way ANOVA with Dunnett's multiple comparisons test post hoc

Table S5. SNPs in RsbU* determined by Whole-Genome Sequencing.

Location (bp)	Locus Tag	Gene function	Change	Coverage at site	Possible Effect
52,232	CT674	YscC, type II secretion system protein	G664W	306x, 97.4%	Loses possible turn
119,402	CT727	zntA, metal transporting ATPase	silent, S102	200x, 98.5%	N/A
133,223	IGR	N/A	N/A	226x, 96%	Likely in the promotor region for ribA, may effect expression
137,588	CT734	lipoprotein	silent, P218	208x, 95.2%	N/A
363,948	**CT036**	**hypothetical protein, putative inc**	**G182S**	**291x, 96.9%**	**likely in a beta strand facing host cytosol, no obvious effect**
431,642	**CT094**	**tRNA pseudouridine synthase B**	**silent, L62**	**439x, 97.3%**	**N/A**
435,453	**CT097**	**nusA, transcription termination/antitermination protein**	**A247V**	**411x, 98.5%**	**Likely in beta strand, no obvious effect**
440,207	**IGR**	**N/A**	**N/A**	**543x, 98.3%**	**Between 2 diverging genes, potential expression effects**
448,523	CT109	hypothetical protein	A37V	420x, 97.9%	no obvious effects
449,796	CT110	GroEL, chaperonin	silent, L200	385x, 96.9%	N/A
452,840	**IGR**	**N/A**	**N/A**	**443x, 97.7%**	**Between divergent genes, possible small RNA or regulatory region**
477,508	CT138	microsomal dipeptidase	P70S	382x, 98.4%	potential lengthening of a turn region
509270	CT163	hypothetical protein	Q204*	688x, 98.75	Major truncation event, contains about 1/3 extracellular domain, in PZ
547175	**CT204**	**ybhI, sodium:sulfate symporter**	**S31F**	**455x, 98.2%**	**no obvious effect, in extracellular domain**
609746	**CT259**	**protein phosphatase**	**G105E**	**583x, 99.1%**	**possible addition of a-helix, not in active site**
616,827	CT266	hypothetical protein	silent, E105	572x, 97.0%	N/A
630,626	**CT281**	**nqrE, Na(+)-translocating NADH-quinone reductase subunit E**	**silent, L3**	**533x, 97.7%**	**N/A**
637,039	CT286	clpC, ATP-dependent Clp protease ATP-binding subunit	R204C	631x, 98.9%	no obvious effects
638,353	CT286	clpC, ATP-dependent Clp protease ATP-binding subunit	F642L	455x, 98.0%	no obvious effects
672,564	CT314	rpoC, DNA-directed RNA polymerase subunit beta'	E195K	394x, 97.7%	no obvious effects
675,519	CT315	rpoB, DNA-directed RNA polymerase subunit beta	H471Y	403x, 98.5%	no obvious effects
689,582	CT329	xseA, exodeoxyribonuclease VII large subunit	silent, Y279	486x, 99.0%	N/A
693,265	CT332	pykF, pyruvate kinase	S158N	372x,98.7%	possible introduction of a turn
789,731	CT411	lpxB, lipid-A-disaccharide synthase	L601F	583x, 97.4%	no obvious effects
798,820	CT414	pmpC	G157E	395x, 97.7%	possible shorter helix
847,452	CT454	argS, arginine--tRNA ligase	P412L	341x, 97.55	no obvious effects
869381	**CT476**	**hypothetical protein, ywqK antitoxin module subunit domain (E=3.23e-57)**	**C8Y**	**293x, 97.3%**	**maybe interferes with predicted signal cleavage site, interupts a-helix**
872,210	**CT478**	**oppC2, oligopeptide ABC transporter permease**	**R127W**	**429x, 97.9%**	**no obvious effects**
888,891	**CT493**	**polA, DNA polymerase I**	**G310E**	**394x, 98.7%**	**possibly extends helix**
906,093	**CT510**	**secY, preprotein translocase subunit**	**L146F**	**522x, 97.5%**	**no obvious effects**
929,717	**IGR**	**N/A**	**N/A**	**390x, 96.9%**	**possibly effects expression of CT544**
1,014,167	**CT615**	**rpoD, sigma-66**	**D50N**	**443x, 98.6x**	**no obvious effects**

Lines in bold indicate those SNPs that maintain the wild-type version of the gene in the RsbU+ complemented strain.