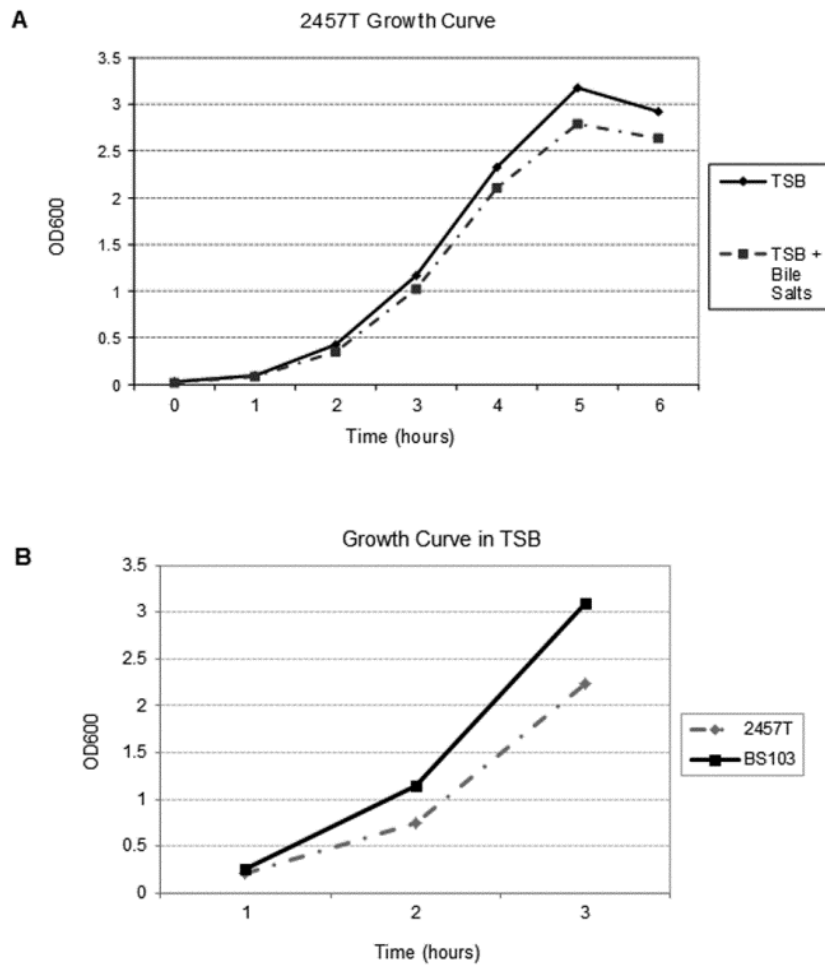
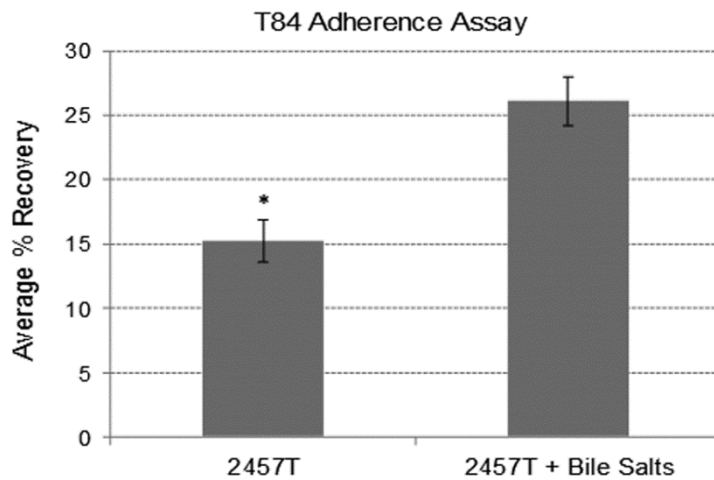


Supplementary Data



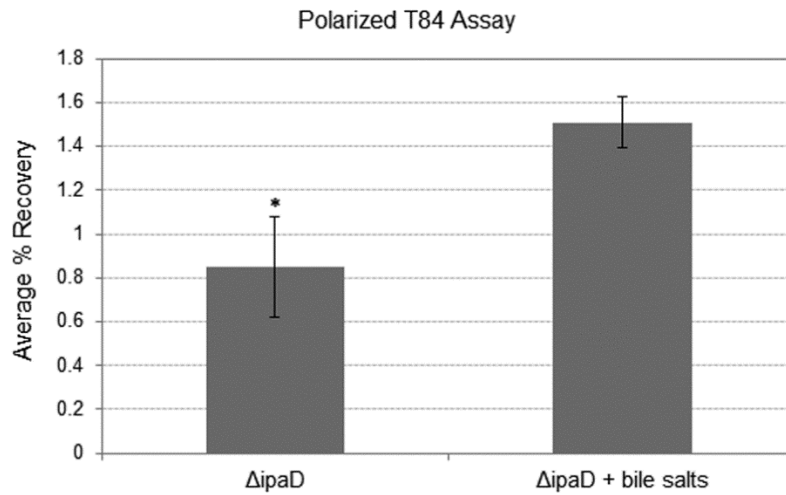
Supplementary Figure 1A: Exposure to bile salts does not significantly alter the growth of *Shigella flexneri*. An example of a growth curve is provided. 2457T was subcultured in tryptic soy broth (TSB) alone or with 0.4% w/v bile salts. Growth was measured over the course of 6 hours by taking optical density readings at 600 nm (OD₆₀₀). Bacteria exposed to bile salts grew at the same rate as bacteria subcultured in TSB alone, especially during mid-log phase. The data indicated that exposure to bile salts did not affect the growth rate of *S. flexneri*.

Supplementary Figure 1B: The virulence plasmid-cured strain BS103 grows faster than wildtype 2457T. An example of a growth curve is provided in which both strains were subcultured in TSB.

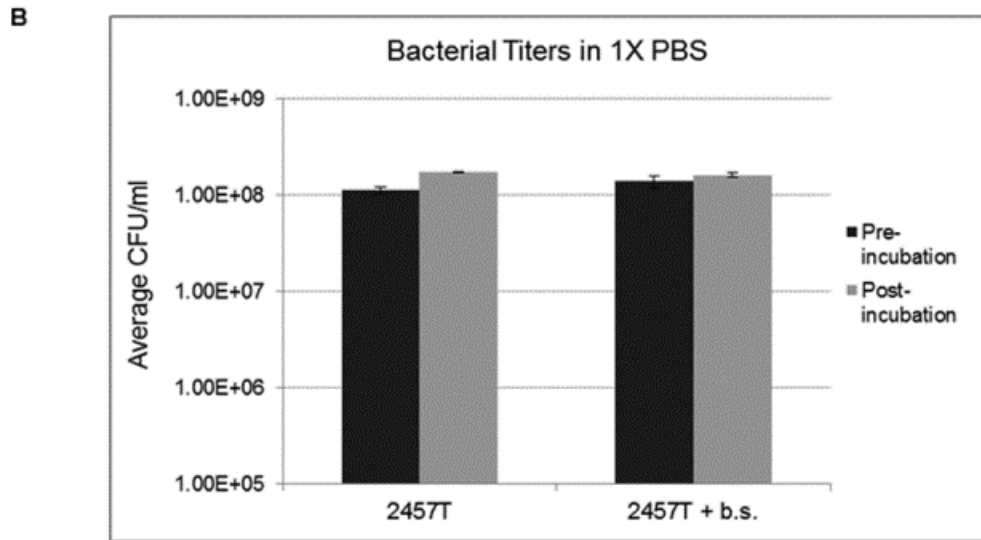
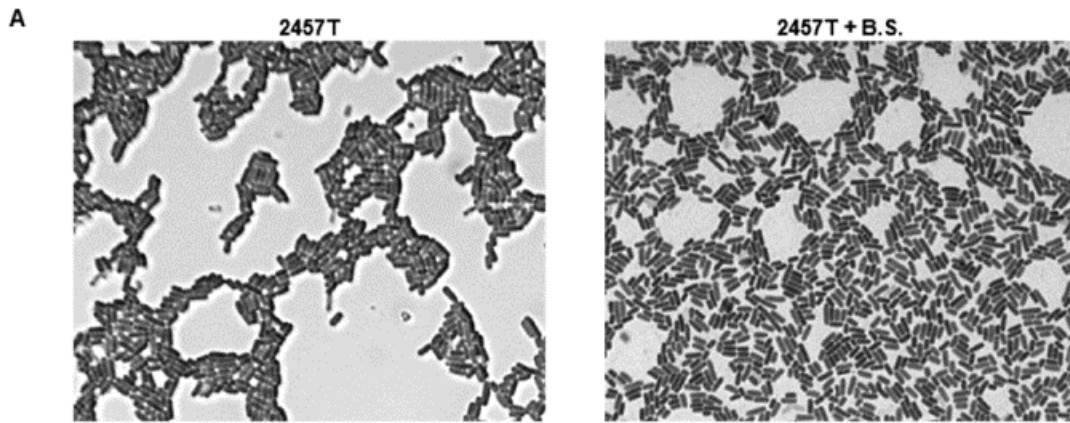


Supplementary Figure 2: Bile salts increase adherence of 2457T to a monolayer of T84 cells.

Bacteria were subcultured with or without 0.4% w/v bile salts and applied to semi-confluent monolayers of T84 cells for 3 hours. Wildtype *S. flexneri* strain 2457T consistently had a significant increase in adherence after being exposed to the bile salts during subculture. Analysis was performed for three independent experiments. Plotted is the average percent (%) recovery of the adherent bacteria +/- the standard error. * = p -value <0.01, as determined by the student's T-test.

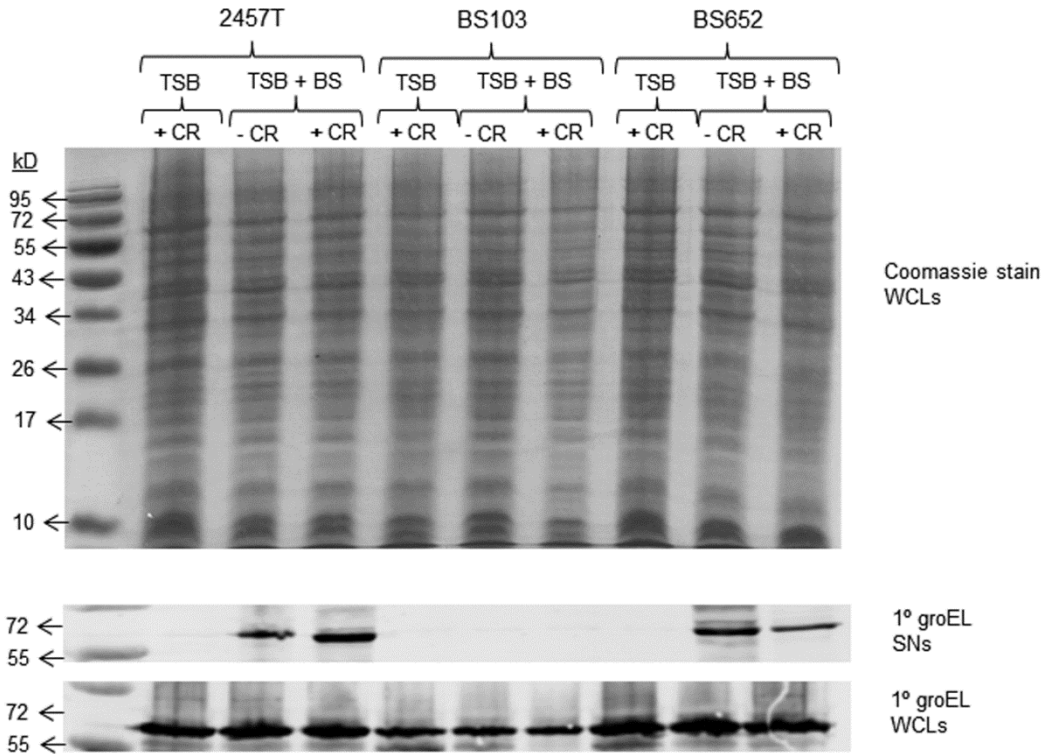


Supplementary Figure 3: The $\Delta ipaD$ mutant has increased adherence to polarized T84 cells after bile salts exposure. Induced adherence was detected despite the fact that this mutation causes induced secretion of T3SS effectors. This mutation was constructed in *S. flexneri* serotype 5 strain M90T. * = *p*-value of 0.028, as determined by the student's T-test.

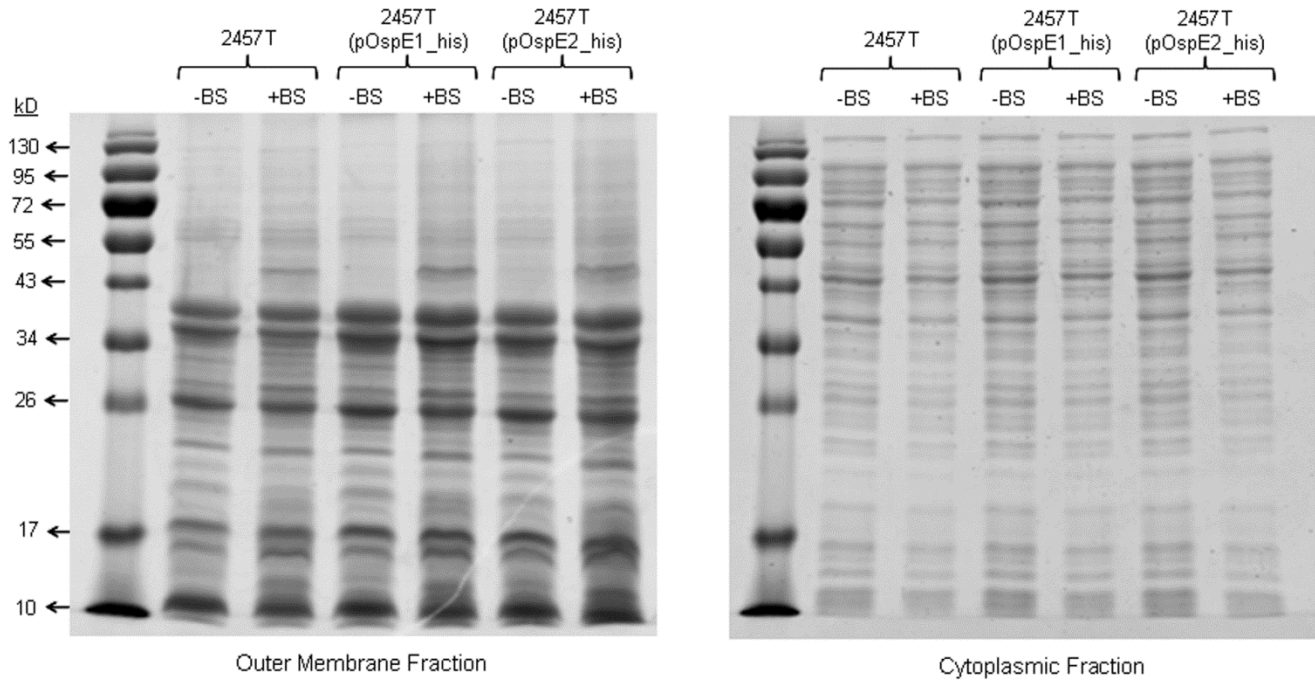


Supplementary Figure 4: Bile salts do not cause bacterial lysis. A: The bacteria were prepared as described in the Experimental Procedures for the protein secretion, immunofluorescence, and outer membrane localization assays. The bacteria were pelleted and Giemsa-stained to analyze bacterial cell morphology. Bacteria subcultured in both TSB with or without bile salts had a normal shape.

B: Bacterial viability was demonstrated by determining the bacterial titers (colony forming units (CFU)/ml) before and after incubation at 37°C for 1 hour in 1X PBS. The average recovered CFU/ml is plotted +/- the standard error. The data indicated that no significant lysis of the bacteria occurred.



Supplementary Figure 5: The induced protein secretion that occurs following bile salts exposure is not due to lysis of the bacteria. The CR assay supernatants from Figure 4 were probed with an antibody that recognizes the cytoplasmic protein GroEL. While GroEL was detected in the supernatants of 2457T and BS652 following bile salts exposure, the protein was not detected in the supernatants of the plasmid-cured strain BS103. The Coomassie-stained whole cell lysates and Western blot for GroEL are also provided.



Supplementary Figure 6: Protein profile of the outer membrane and cytoplasmic proteins obtained from ultracentrifugation of bacterial pellets. The outer membrane (OM) fraction is on the left while the cytoplasmic fraction is on the right. The predominant OM proteins obtained are localized around 20 kD and between 25 to 45 kD. The protein profile agrees with previous OM preparations of *S. flexneri*.

Supplementary Table 1. Gene Expression Differences with Bile Salts

Condition	Number of genes				
Increased with Bile Salts	51				
Decreased with Bile Salts	49				

Increased					
Affy_ID	Bile	No Bile	Fold Change	p-value (t-test)	Annotation
b3238_s_at	11.89338131	9.241896533	6.283135874	0.00075986	yhcN; hypothetical protein
V5882_s_at	11.16186806	9.365784449	3.472762168	0.026755035	sodA; superoxide dismutase
A0384_s_at	7.740291355	5.964157753	3.425070307	0.000473746	S2558 = hypothetical protein
A2361_s_at	12.96499445	11.19408553	3.412688939	0.015980829	groEL; chaperonin GroEL ; K04077
V1059_s_at	11.36918459	9.735085829	3.10393589	3.45E-06	acrB; acridine efflux pump
b4142_s_at	13.38676818	11.7945722	3.015079364	0.017288551	S4563; co-chaperonin GroES
ECP_0524_s_at	13.10846068	11.55175855	2.941806039	1.94E-05	acrA; acriflavine resistance protein A ; K03585 membrane fusion protein
N3131_x_at	5.899753118	4.361807791	2.903806514	0.015322546	feoC; ferrous iron transport protein C
V5247_s_at	5.715650963	4.194836461	2.869530089	0.02581015	putative DNA-binding transcriptional regulator yhjC
V1345_s_at	11.41689535	9.901857735	2.858062802	0.024610015	dnaK; chaperone protein DnaK; K04043 molecular chaperone DnaK
A4409_s_at	9.322964834	7.828913669	2.816788349	0.001574581	fumC; fumarate hydratase (EC:4.2.1.2); K01679
SDY_P046_s_at	11.95591012	10.47935119	2.78284186	0.001748628	ospE1, ospE2
SD1_0506_x_at	6.328387215	4.867686562	2.752420038	0.013069522	yhgG; hypothetical protein ; K07490 ferrous iron transport protein C
b2141_s_at	9.341895935	7.891238751	2.733325329	0.043520369	hypothetical protein ; K06518 holin-like protein
J2890_s_at	9.530872009	8.105862133	2.685163387	0.001543212	nitroreductase S0847
V5836_s_at	9.527119852	8.116409624	2.658680154	0.046024159	hslU; ATP-dependent protease ATP-binding subunit
EC536_1048_s_at	12.57964756	11.1785387	2.641044957	0.044179779	putative glutamate dehydrogenase
V5908_s_at	7.378390553	5.989937315	2.61797848	0.00604376	frvX; putative fructose-specific phosphotransferase system protein FrvX
A2145_s_at	11.89016846	10.50590518	2.610386217	0.026051502	hslU; ATP-dependent protease ATP-binding subunit HslU
G2276_s_at	10.02210896	8.648092115	2.591912187	0.001045958	mdaA; nitroreductase A ; K10678 nitroreductase
A3523_s_at	10.61286551	9.27334682	2.53066877	0.000329718	ybjC; hypothetical protein
V5594_at	9.182022346	7.859617491	2.500826299	0.002849686	hypothetical protein
V5833_s_at	12.14027579	10.81961525	2.497804451	0.032638352	hslV; ATP-dependent protease peptidase subunit
A1703_s_at	5.426804491	4.112141583	2.487442037	0.000383345	treF; trehalase (EC:3.2.1.28); K01194
SF1779_s_at	10.8118276	9.500645894	2.481447113	0.017258065	hypothetical protein S1495
EC869_0338_s_at	5.750113751	4.441524987	2.476991236	0.023265408	ferrous iron transport protein feoB
H1461_s_at	6.995722777	5.7021473	2.451348288	0.002170631	hypothetical protein S1495
V0964_s_at	12.46527477	11.17886583	2.439201482	9.02E-05	hypothetical protein S3465
SB5_2172_s_at	9.513547017	8.244865137	2.409413285	0.001105965	hypothetical protein S2452

F1059_at	6.883998151	5.63920035	2.369853375	0.018766839	hypothetical protein; NO_Kegg_data
SBO_1654_s_at	9.371176785	8.143770554	2.341456491	0.008873785	hypothetical protein S1495
SB5_2171_s_at	11.28710369	10.08435092	2.301784501	0.002389386	inaA; hypothetical protein
EC869_3291_s_at	10.52305953	9.344093034	2.264145229	0.010047002	3-oxoacyl-(acyl carrier protein) synthase
SBO_1749_s_at	8.93761928	7.760097246	2.261879439	0.043549145	ycjX; putative YcjX (EC:2.1.-.-); K06918
SD1_2172_s_at	8.874930509	7.698983724	2.259411086	0.011037027	fabB; 3-oxoacyl-(acyl carrier protein) synthase I
A2146_s_at	12.90792059	11.75004735	2.231282585	0.01850293	hslV; ATP-dependent protease peptidase subunit
b3236_s_at	11.43903747	10.28878837	2.219522136	0.030582084	mdh; malate dehydrogenase (EC:1.1.1.37); K00026 malate dehydrogenase [EC:1.1.1.37]
SDY_4085_s_at	6.754055152	5.621591234	2.192328392	0.01013317	glutamate transport protein gltS
A3363_s_at	9.376647174	8.248111538	2.18636707	0.01426047	sdhB; succinate dehydrogenase iron-sulfur subunit (EC:1.3.99.1); K00240 succinate dehydrogenase iron-sulfur protein [EC:1.3.99.1]
EC869_5027_s_at	9.368195546	8.250548153	2.16992833	0.017635438	sdhA; succinate dehydrogenase flavoprotein subunit; K00239
A1888_s_at	10.47566153	9.3708929	2.150643846	0.018593741	ibpA; heat shock protein IbpA ; K04080 molecular chaperone IbpA
A4665_s_at	10.89396878	9.805140464	2.127012204	0.023412699	eda; keto-hydroxyglutarate-aldolase/keto-deoxy- phosphogluconate aldolase (EC:4.1.2.14 4.1.3.16); K01625 2-dehydro-3-deoxyphosphogluconate aldolase / 4-hydroxy-2-oxoglutarate aldolase [EC:4.1.2.14 4.1.3.16]
A1588_s_at	10.77586188	9.703400151	2.103018766	0.022674728	yhgl; putative DNA uptake protein ; K07400 Fe/S biogenesis protein NfuA
b3441_s_at	7.813471036	6.743842726	2.098892549	0.015848732	putative acetyltransferase YhhY ; K03825 putative acetyltransferase [EC:2.3.1.-]
V5109_s_at	6.253263274	5.18470699	2.0973335	0.01825132	malS; periplasmic alpha-amylase precursor; K01176 alpha-amylase [EC:3.2.1.1]
EC536_0230_s_at	5.700737443	4.647555203	2.075101982	0.013999239	yfeO; putative ion channel protein
V6436_x_at	7.941270358	6.894613613	2.065737224	0.037294055	hypothetical protein S1410
A4118_s_at	8.567344598	7.530494202	2.051743517	0.045590145	hypothetical protein S1410
b3207_s_at	12.79397427	11.77678903	2.023966261	0.000167356	yrbL; hypothetical protein
b0492_s_at	9.070914384	8.055521718	2.021453008	0.046428755	ybbN; predicted thioredoxin domain-containing protein ; K05838 putative thioredoxin
EC869_5024_x_at	7.220949116	6.21577251	2.007189189	0.019316711	sdhA; succinate dehydrogenase flavoprotein subunit ; K00239

Decreased

Affy_ID	Bile	No Bile	Fold Change	p-value (t-test)	Annotation
V5274_s_at	3.880931324	5.891527752	-4.029487696	0.000372394	hypothetical protein
b3512_s_at	5.01061716	6.889082905	-3.676838335	0.00515398	yhiE; hypothetical protein
EC869_2081_x_at	6.119992984	7.734365095	-3.061783156	0.003495379	ccmF; cytochrome c-type biogenesis protein ; K02198
F2824_s_at	4.223282457	5.785786853	-2.95366129	0.026734404	putative enzyme; K06889
S1481_at	5.561612611	7.123085044	-2.951549287	0.037463794	sokB = S1481; IS103 orf

A1183_s_at	7.92714577	9.471151493	-2.916030325	0.007820975	hybG; hydrogenase 2 accessory protein HypG ; K04653
V1312_s_at	3.616276946	5.152679132	-2.900702194	0.043158598	phosphoglycerate mutase ; K01834
S1762_s_at	4.665645657	6.195549569	-2.887666055	0.024905766	putative transporter; K06956
V0189_x_at	4.602752257	6.064862992	-2.755111555	0.019788533	nrfG; formate-dependent nitrite reductase complex subunit
EC536_3469_s_at	8.902851599	10.36405028	-2.753370352	0.0092344	ccmE; cytochrome c-type biogenesis protein CcmE ; K02197
F2496_s_at	8.808115324	10.22467739	-2.669486154	0.03381573	ccmA; cytochrome c biogenesis protein CcmA ; K02193
V1726_s_at	9.102852512	10.48326015	-2.603419213	0.016781277	ccmE; cytochrome c-type biogenesis protein CcmE ; K02197
F2493_s_at	9.490126433	10.8518449	-2.569911129	0.020159885	ccmC; heme exporter protein C ; K02195
A0241_s_at	8.163029602	9.511928312	-2.54717611	0.013194955	ccmF; heme lyase, CcmF subunit; K02198
b2199_s_at	8.42896917	9.768406635	-2.530526296	0.012142961	ccmC; heme exporter protein C ; K02195
EC869_2075_s_at	9.097887286	10.43501247	-2.526473742	0.023190903	ccmE; cytochrome c-type biogenesis protein CcmE ; K02197
A0246_s_at	9.357965278	10.69495978	-2.526244905	0.027058753	ccmA; cytochrome c biogenesis protein CcmA ; K02193
c3620_s_at	8.57434894	9.889302147	-2.48794261	0.032291124	putative secreted autotransporter toxin S3965
V2998_s_at	8.878693389	10.18901723	-2.479972019	0.002380227	evgS; hybrid sensory histidine kinase
V1722_s_at	9.10194195	10.39659195	-2.453174732	0.025231561	ccmB; heme exporter protein B ; K02194
SB5_2059_s_at	8.196709188	9.456856915	-2.395202657	0.009176035	evgS; hybrid sensory histidine kinase
EC869_4712_s_at	5.977258274	7.233390336	-2.388545008	0.029064788	hypothetical protein
A1184_s_at	8.636220483	9.859609811	-2.334946231	0.023318945	hybF; HybF protein; K04651
SDY_2117_s_at	5.661990041	6.854321219	-2.285217017	0.006726165	putative bacteriophage protein S0691
V1729_s_at	9.78561179	10.96280559	-2.261364895	0.025968744	cytochrome c-type biogenesis protein
EC869_2076_s_at	9.395195543	10.56857101	-2.255387721	0.016258205	ccmF; cytochrome c-type biogenesis protein ; K02198
EC869_2084_s_at	9.097565661	10.26867837	-2.251853092	0.047299796	dsbE; disulfide oxidoreductase ; K02199
V4544_x_at	5.954740259	7.119823328	-2.24246127	0.03043683	argI; ornithine carbamoyltransferase subunit I
A4083_s_at	6.39372548	7.557849646	-2.24097129	0.017666355	hypothetical protein S1376; ycjD
SD1_3403_s_at	5.804406499	6.95912328	-2.226406122	0.03987058	glutamate and aspartate transporter subunit ; K10001
EC869_5555_s_at	6.614653846	7.763387676	-2.217192189	0.01235153	ornithine carbamoyltransferase 1
Z4493_s_at	4.177372423	5.317649366	-2.204233321	0.044205839	agaC; N-acetylgalactosamine-specific PTS system transporter subunit IIC
A0243_s_at	8.746430337	9.885938775	-2.203059465	0.027381936	ccmD; heme exporter protein C ; K02196
A0240_s_at	8.997117664	10.13505806	-2.20066629	0.040945632	dsbE; disulfide oxidoreductase ; K02199
SB5_1951_s_at	7.571256025	8.686675201	-2.166579499	0.039956996	S2797; yfhL hypothetical protein
O2CoIV74_at	3.760037207	4.838747344	-2.11214684	0.027463077	ISSfI4 ORF3 ; K07484 transposase
ECP_2234_s_at	8.980594011	10.05891304	-2.111574329	0.047338116	cytochrome c-type biogenesis protein CcmH precursor; K02200
SD1_2150_s_at	5.648741242	6.726901517	-2.11134198	0.001912015	S0592; ybgQ; putative outer membrane protein
EC536_0524_x_at	8.46261721	9.537002886	-2.105825185	0.036723139	hybE; hydrogenase 2-specific chaperone
S0688_s_at	3.957037152	5.028067933	-2.100933913	0.015461997	hypothetical protein; S0688
SS5_4161_s_at	9.288658067	10.35561266	-2.095006311	0.009799359	putative bacteriophage protein; S0693
SS5_4621_s_at	6.988845765	8.046495086	-2.08153717	0.046271452	yihF; hypothetical protein
EC869_7558_s_at	6.504189076	7.5582349	-2.076344491	0.001923349	dcuC; C4-dicarboxylate transporter DcuC ; K03326
A3108_s_at	3.779970621	4.827761866	-2.067362308	0.002970713	glnK; nitrogen regulatory protein P-II 2 ; K04752

SS5_1921_s_at	5.271211014	6.314800285	-2.061349697	0.000310492	molR_1; molybdate metabolism regulator, first fragment
V2689_s_at	9.614462909	10.64579722	-2.043913743	0.040027718	hybD; hydrogenase 2 maturation endopeptidase ; K08567
UT189_C2904_s_at	3.650827694	4.669953653	-2.026690738	0.029184225	conserved hypothetical protein, putative Z3866 protein
SD1_0026_at	3.182008664	4.193741699	-2.016331761	0.031721717	asnA; asparagine synthetase AsnA
A0247_s_at	11.06481688	12.07397808	-2.012740527	0.038923611	hypothetical protein; S2416; napC

Supplementary Table 2. Primers used in this study.

Purpose	Forward Primer		Reverse Primer	
	Name	Sequence	Name	Sequence
Deletions				
Amplify <i>aphA-3</i> for <i>ospE1</i> deletion	E1kanF	5' – CTTGTCTTCCACAAAAACAAGA AAATATCATCCTTGAAGTATCAAA TCATATGAATACCTCCTTAGTTCC – 3'	E1kanR	5' – CTTTGTTTTTTCATCAATTCCGGCTT CATTATCTTTGTCAACCATTGCATTGTG TAGGCTGGAGCTGCTTC – 3'
Confirm Δ <i>ospE1</i>	E1F	5' – AGTCATCGGCTCCTTCATTG – 3'	E1R	5' – AGGTATTTAAGGTCATTGCG – 3'
Amplify <i>cat</i> for <i>ospE2</i> deletion	E2catF	5' – TTAATAAAATATAACATAAGGA AGCAATATGCTTACACAACTATATTC CTGTGTAGGCTGGAGCTGCTTC – 3'	E2catR	5' – TGGAGCTTAAACGAGTCTGAAATTAA CTTCAGAAATCAGAATACCGTTGGCATAT GAATACCTCCTTAG – 3'
Confirm Δ <i>ospE2</i>	E2F	5' – TCTGGCTCCACATCTCATCG – 3'	E2R	5' – ACCGTATCGAGGATAGATGG – 3'
qRT-PCR				
<i>rpoA</i>	rpoAF	5' – AGCGCGGTCTGTGGTTATGTG – 3'	rpoAR	5' – CGCTGCTTCAACATTGTAGG – 3'
<i>ospE1/2</i>	ospEF	5' – CACAACTATATCCCTTGC – 3'	ospER	5' – TTTCAACGTTTTTCGTCCGGT – 3'
<i>ccmF</i>	ccmFF	5' – TCGGCATTGTGCTCGGTTCC – 3'	ccmFR	5' – TGACCGCCAGTGAGTGCATC – 3'
<i>yhcN</i>	yhcNF	5' – GCTGCCGACTCCATTGATGC – 3'	yhcNR	5' – CCGTTGCGCCTTTCTCTTCC – 3'
<i>ybgQ</i>	ybgQF	5' – GGTGACGATCAGTCAATCAG – 3'	ybgQR	5' – TTCACCCGACCATCTTCTTC – 3'
Cloning				
Amplify <i>ospE1</i> for pCSF1	E1F	5' – AGTCATCGGCTCCTTCATTG – 3'	E1R	5' – AGGTATTTAAGGTCATTGCG – 3'
Amplify <i>ospE2</i> for pCSF2	E2F	5' – TCTGGCTCCACATCTCATCG – 3'	E2R	5' – ACCGTATCGAGGATAGATGG – 3'
Amplify <i>ospE</i> for pOspE ⁺	E3F	5' – ATGCGGATCCCAGTAACTTC ATTTAGCCGAC – 3'	ER2	5' – TCAGAAATCAGAATACCGTTGG – 3'
Amplify <i>ospE1</i> for pCSF3	E3F	5' – ATGCGGATCCCAGTAACTTC ATTTAGCCGAC – 3'	EhisR	5' – TCAATGATGATGATGATGATGATG GAAATCAGAATACCGTTGGC – 3'

Amplify
ospE2 for
pCSF4

E3F

5' – ATGCGGATCCCAGTAACTTC
ATTTAGCCGAC – 3'

EhisR

5' – TCAATGATGATGATGATGATGATG
GAAATCAGAATACCGTTGGC – 3'
