

**Table S2A. List of ORFs with nonsynonymous changes from the reference strain MG1655 to ER2796 (disrupted ORFs).**

MG1655 Gene	MG1655 Locus	ER2796 Gene	ER2796 Locus	ER2796 Start.Stop	Disruption	Intervening ORFs	MG1655 Functional Description
fhuA	b0150	fhuA	ER2796_149/151	167484..171063	IS2 insertion	ER2796_148, ER2796_150	ferrichrome outer membrane transporter
rclA	b0304	rclA	ER2796_311/-	321899..319236	IS10 insertion	ER2796_310	putative pyridine nucleotide-disulfide oxidoreductase
lacZ	b0344	lacZ	ER2796_352	365759..364971	deletion	—	beta-D-galactosidase
ybcV	b0558	ybcV	—	575649..575239	deletion	—	DLP12 prophage; putative protein
nohD	b0560	nohD	ER2796_-/567	576889..578211	IS1 insertion	ER2796_565, ER2796_566	DLP12 prophage; DNA packaging protein
yliE	b0833	yliE	ER2796_-/851	869811..872158	frameshift	—	putative membrane-anchored cyclic-di-GMP phosphodiesterase
rssB	b1235	rssB	ER2796_1251/1252	1271872..1272885	nonsense	—	response regulator binding RpoS to initiate proteolysis by ClpXP; required for the PcnB-degradosome interaction during stationary phase
yciT	b1284	yciT	ER2796_1307/1306	1324776..1324028	frameshift	—	global regulator of transcription; DeoR family
ydbH	b1381	ydbH	ER2796_1409/-	1423480..1426119	nonsense	—	hypothetical protein
btuD	b1709	btuD	ER2796_1752/1751	1775323..1774575	frameshift	—	vitamin B12 transporter subunit : ATP-binding component of ABC superfamily
htpX	b1829	htpX	ER2796_1879/1878	1894341..1893460	nonsense	—	putative endopeptidase
rsmF	b1835	rsmF	ER2796_-1885/1886	1901988..1903427	nonsense	—	16S rRNA m(5)C1407 methyltransferase, SAM-dependent
dcm	b1961	dcm	ER2796_2013/2012	2013306..2011888	nonsense	—	DNA cytosine methyltransferase
yedZ	b1972	yedZ	ER2796_2025/2035	2021472..2031263	Tn10 insertion	ER2796_2026 to _2034	inner membrane heme subunit for periplasmic YedYZ reductase
hisG	b2019	hisG	ER2796_2082	2080337..2081200	deletion	—	ATP phosphoribosyltransferase
rfbD	b2040	rfbD	ER2796_2102/-	2102084..2101186	frameshift	—	dTDP-4-dehydrorhamnose reductase subunit, NAD(P)-binding, of dTDP-L-rhamnose synthase
mglA	b2149	mglA	ER2796_2216/-	2230172..2227875	IS1 insertion	ER2796_2215	fused methyl-galactoside transporter subunits of ABC superfamily: ATP-binding components
rhmD	b2247	rhmD	ER2796_2322/2321	2352296..2351092	frameshift	—	L-rhamnose dehydratase
eutA	b2451	eutA	ER2796_2526	2550977..2549574	deletion	—	reactivating factor for ethanolamine ammonia lyase
luxS	b2687	luxS	ER2796_2763/2762	2798833..2798319	frameshift	—	S-ribosylhomocysteine lyase
hycC	b2723	hycC	ER2796_2803/2802	2833341..2831515	nonsense	—	hydrogenase 3, membrane subunit
rpoS	b2741	rpoS	ER2796_-/2821	2851651..2850659	nonsense	—	RNA polymerase, sigma S (sigma 38) factor
cptB	b2897	cptB	ER2796_2983/-	3025170..3024904	nonsense	—	antitoxin of CptAB toxin-antitoxin pair
galP	b2943	galP	ER2796_3029/3030	3072384..3073778	nonsense	—	D-galactose transporter
ttdB	b3062	ttdB	ER2796_3151/3152	3191471..3192075	frameshift	—	L-tartrate dehydratase, beta subunit
tdcD	b3115	tdcD	ER2796_3208/3206	3248958..3246551	IS5 insertion	ER2796_3207	propionate kinase/acetate kinase C, anaerobic
argG	b3172	argG	ER2796_3265/3266	3303935..3305277	frameshift	—	argininosuccinate synthetase
dam	b3387	dam	ER2796_-/3474	348593..3484024	deletion, KanR insertion	ER2796_3475	DNA adenine methyltransferase
xylF	b3566	xylF	ER2796_3671/3673	3702011..3703780	IS1 insertion	ER2796_3672	D-xylene transporter subunit
mtlA	b3599	mtlA	ER2796_3708/3709	3743938..3745849	frameshift	—	fused mannitol-specific PTS enzymes: IIA components/IB components/IIC components
rph	b3643	rph	ER2796_3754/-	3788205..3787518	frameshift	—	defective ribonuclease PH
metB	b3939	metB	ER2796_-/4061	4100328..4101486	frameshift	—	cystathione gamma-synthase, PLP-dependent
opgB	b4359	opgB	ER2796_4466	4516451..4514439	deletion, IS10 insertion	—	phosphoglycerol transferases I and II

**Table S2B. List of ORFs with nonsynonymous changes from the reference strain MG1655 to ER2796 (ORFs with missense mutations).**

MG1655 Gene	MG1655 Locus	ER2796 Gene	ER2796 Locus	Codon Change(s)	Consequence(s)	MG1655 Functional Description
rpsT	b0023	rpsT	ER2796_18	GCC -> GTC	A11V	30S ribosomal subunit protein S20
ileS	b0026	ileS	ER2796_20	GAT -> AAT	D510N	isoleucyl-tRNA synthetase
yadV	b0140	yadV	ER2796_137	GTA -> GCA	V45A	putative periplasmic pilin chaperone
yafJ	b0223	yafJ	ER2796_228	GCG -> AGC	G45S	putative amidotransfase
ecpD	b0290	ecpD	ER2796_297	TCC -> TTT	S202F	putative receptor
cynX	b0341	cynX	ER2796_349	GGC -> GAC	G348D	putative cyanate transporter
clpX	b0438	clpX	ER2796_448	GAT -> AAT	D255N	ATPase and specificity subunit of ClpX-ClpP ATP-dependent serine protease
lon	b0439	lon	ER2796_449	TCT -> TTT	S182F	DNA-binding ATP-dependent protease La
yibA	b0515	yibA	ER2796_524	TAA -> CAA	stop262Q	hypothetical protein
entA	b0596	entA	ER2796_607	AAT -> GAT	N7D	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase
ybiT	b0820	ybiT	ER2796_838	CCT -> TCT; ACG -> AAG	P158S; T360K	fused predicted transporter subunits of ABC superfamily: ATP-binding components
rimK	b0852	rimK	ER2796_870	GAA -> AAA	E178K	ribosomal protein S6 modification protein
rlmC	b0859	rlmC	ER2796_878	CGT -> CAT	R143H	23S rRNA m(5)U747 methyltransferase, SAM-dependent
rpsA	b0911	rpsA	ER2796_932	CGT -> TGT	R171C	30S ribosomal subunit protein S1
smtA	b0921	smtA	ER2796_942	TCC -> TTC	S14F	putative S-adenosyl-L-methionine-dependent methyltransferase
uup	b0949	uup	ER2796_972	GAA -> AAA	E553K	fused predicted transporter subunits of ABC superfamily: ATP-binding components
yceF	b1087	yceF	ER2796_1115	GTT -> TTT	V173F	Maf-like protein
ptsG	b1101	ptsG	ER2796_1130	GTC -> TTC	V12F	fused glucose-specific PTS enzymes: IIB component/IIC component
ycfS	b1113	ycfS	ER2796_1142	CTG -> CCG	L180P	L,D-transpeptidase linking Lpp to murein
icd	b1136	icd	ER2796_1166	GAT -> GAA	D398E	e14 prophage; isocitrate dehydrogenase, specific for NADP+
nhaB	b1186	nhaB	ER2796_1197	TCT -> TTT	S350F	sodium:proton antiporter
oppA	b1243	oppA	ER2796_1262	AAT -> TAT	N271Y	oligopeptide transporter subunit
oppF	b1247	oppF	ER2796_1266	TCC -> GCC	S325A	oligopeptide transporter subunit
yciB	b1254	yciB	ER2796_1274	GAG -> AAG	E72K	putative inner membrane protein
trpE	b1264	trpE	ER2796_1284	GGT -> GAT	G454D	component I of anthranilate synthase
acnA	b1276	acnA	ER2796_1297	AGC -> GGC	S522G	aconitate hydratase 1
gmr	b1285	gmr	ER2796_1309	GCT -> ACT	A166T	cyclic-di-GMP phosphodiesterase; csgD regulator; modulator of Rnase II stability
sapA	b1294	sapA	ER2796_1318	CCC -> CTC	P146L	antimicrobial peptide transport ABC transporter periplasmic binding protein
puuP	b1296	puuP	ER2796_1320	TAT -> TGT; GTA -> ATA	Y110C; V139I	putrescine importer
puuE	b1302	puuE	ER2796_1328	ACC -> ATC	T73I	GABA aminotransferase, PLP-dependent
pspF	b1303	pspF	ER2796_1329	TTG -> TTT	L229F	DNA-binding transcriptional activator
ycjN	b1310	ycjN	ER2796_1336	TCA -> TTA	S4L	putative sugar transporter subunit: periplasmic-binding component of ABC superfamily
ycjP	b1312	ycjP	ER2796_1338	GGC -> TGC	G137C	putative sugar transporter subunit: membrane component of ABC superfamily
stfR	b1372	stfR	ER2796_1398	CCG -> TCG	P68S	Rac prophage; predicted tail fiber protein
hrpA	b1413	hrpA	ER2796_1443	CCA -> TCA	P1081S	putative ATP-dependent helicase
fdnH	b1475	fdnH	ER2796_1510	AAA -> ATA	K177I	formate dehydrogenase-N, Fe-S (beta) subunit, nitrate-inducible
adhP	b1478	adhP	ER2796_1513	GGT -> GTT	G70V	ethanol-active dehydrogenase/acetaldehyde-active reductase
lsrR	b1512	lsrR	ER2796_1549	GTG -> ATG	V47M	lsr operon transcriptional repressor
yneE	b1520	yneE	ER2796_1557	GAT -> GGT	D298G	putative inner membrane protein, bestrophin family
ydfK	b1544	ydfK	ER2796_1584	AAA -> ACA	K51T	cold shock protein, function unknown, Qin prophage
ydfU	b1560	ydfU	ER2796_1601	CTG -> CAG	L209Q	Qin prophage; putative protein
ydfC	b1573	ydfC	ER2796_1610	TCC -> TTC	S47F	conserved protein, Qin prophage
ydfD	b1576	ydfD	ER2796_1613	CCG -> TCG	P60S	Qin prophage; putative protein
asr	b1597	asr	ER2796_1634	ACC -> ATC	T26I	acid shock-inducible periplasmic protein
ydgC	b1607	ydgC	ER2796_1643	CTT -> TTT	L40F	inner membrane protein, GlpM family
ydiJ	b1687	ydiJ	ER2796_1729	CGC -> TGC	R693C	putative FAD-linked oxidoreductase
ydiY	b1722	ydiY	ER2796_1766	TTG -> TCG	L4S	putative outer membrane protein, acid-inducible
yoaA	b1808	yoaA	ER2796_1855	GGC -> AGC	G120S	conserved protein with nucleoside triphosphate hydrolase domain
pabB	b1812	pabB	ER2796_1860	CTC -> CCC	L12P	aminodeoxychorismate synthase, subunit I
ftnB	b1902	ftnB	ER2796_1953	GCC -> GTC	A45V	ferritin B, probable ferrous iron reservoir
yedY	b1971	yedY	ER2796_2024	GCC -> GAC	A319D	membrane-anchored, periplasmic TMAO, DMSO reductase

ugd	b2028	ugd	ER2796_2091	ATT -> AAT	I30N	UDP-glucose 6-dehydrogenase
yegH	b2063	yegH	ER2796_2125	GAA -> AAA	E364K	inner membrane protein
mglC	b2148	mglC	ER2796_2214	CCG -> ACG	P217T	methyl-galactoside transporter subunit
setB	b2170	setB	ER2796_2240	GAG -> ATG	E200M	lactose/glucose efflux system
napB	b2203	napB	ER2796_2274	TTT -> TAT	F141Y	nitrate reductase, small, cytochrome C550 subunit, periplasmic
rcsC	b2218	rcsC	ER2796_2290	GAT -> TAT	D267Y	hybrid sensory kinase in two-component regulatory system with RcsB and YojN
nuoM	b2277	nuoM	ER2796_2353	GGT -> GCT	G270A	NADH:ubiquinone oxidoreductase, membrane subunit M
hisP	b2306	hisP	ER2796_2383	GTA -> ATA	V207I	histidine/lysine/arginine/ornithine transporter subunit
mnmC	b2324	mnmC	ER2796_2403	GCG -> ACG	A562T	fused 5-methylaminomethyl-2-thiouridine-forming enzyme methyltransferase and FAD-dependent demodification enzyme
smrB	b2331	smrB	ER2796_2410	ATG -> ATA	M153I	putative DNA endonuclease
yfeO	b2389	yfeO	ER2796_2471	CCG -> CTG	P327L	putative ion channel protein
narQ	b2469	narQ	ER2796_2544	GCA -> GTA	A461V	sensory histidine kinase in two-component regulatory system with NarP (NarL)
yfgI	b2506	yfgI	ER2796_2584	AGC -> AAC	S176N	hypothetical protein
nrdF	b2676	nrdF	ER2796_2749	TCT -> TTT	S211F	ribonucleoside-diphosphate reductase 2, beta subunit, ferritin-like protein
truD	b2745	truD	ER2796_2826	AAC -> ACC	N51T	tRNA(Glu) pseudouridine(13) synthase
pyrG	b2780	pyrG	ER2796_2865	TCT -> TTT	S237F	CTP synthetase
ygdH	b2795	ygdH	ER2796_2882	CCC -> CTT	P79L	conserved protein, UPF0717 family
araE	b2841	araE	ER2796_2930	GGG -> AGG	G87R	arabinose transporter
yqeF	b2844	yqeF	ER2796_2933	TCC -> TTC	S25F	putative acyltransferase
ygeH	b2852	ygeH	ER2796_2941	CTC -> TTC	L374F	predicted transcriptional regulator
ftsP	b3017	ftsP	ER2796_3108	CGC -> TGC	R352C	septal ring component that protects the divisome from stress; multicopy suppressor of ftsI(Ts)
agal	b3141	agal	ER2796_3234	CCA -> TCA	P96S	galactosamine 6-phosphate isomerase
dacB	b3182	dacB	ER2796_3276	GAT -> TAT	D261Y	D-alanyl-D-alanine carboxypeptidase
aaeB	b3240	aaeB	ER2796_3325	ACG -> CCG	T50P	p-hydroxybenzoic acid efflux system component
secY	b3300	secY	ER2796_3386	CCG -> TCG; GCC -> GTC	P40S; A46V	preprotein translocase membrane subunit
rplD	b3319	rplD	ER2796_3405	CCG -> TCG	P89S	50S ribosomal subunit protein L4
rpsL	b3342	rpsL	ER2796_3428	AAA -> ACA; AAA -> CAA	K43T; K88Q	30S ribosomal subunit protein S12
yhfK	b3358	yhfK	ER2796_3445	GCA -> ACA	A656T	conserved inner membrane protein
malT	b3418	malT	ER2796_3508	GTC -> CTC	V655L	DNA-binding transcriptional activator for the mal regulon and maltotriose-ATP-binding protein
nikD	b3479	nikD	ER2796_3570	ATA -> AAA	I122K	nickel transporter subunit
yhhl	b3484	yhhl	ER2796_3574	GAT -> GAA	D56E	putative transposase
yhiJ	b3488	yhiJ	ER2796_3579	AAT -> CTT	N412L	hypothetical protein
gor	b3500	gor	ER2796_3593	GCG -> GTG	A377V	glutathione oxidoreductase
glyQ	b3560	glyQ	ER2796_3664	GAG -> GCG	E48A	glycine tRNA synthetase, alpha subunit
xylB	b3564	xylB	ER2796_3669	GCG -> GTG	A295V	xylulokinase
xylA	b3565	xylA	ER2796_3670	CAC -> TAC	H271Y	D-xylene isomerase
yiaQ	b3581	yiaQ	ER2796_3688	GCT -> ACT	A68T	3-keto-L-gulonate 6-phosphate decarboxylase
secB	b3609	secB	ER2796_3720	TCC -> TTC	S49F	protein export chaperone
waaY	b3625	waaY	ER2796_3736	GAC -> AAC	D27N	lipopolysaccharide core biosynthesis protein
yidE	b3685	yidE	ER2796_3797	GTT -> GCT	V130A	putative transporter
yieK	b3718	yieK	ER2796_3830	GAA -> AAA	E67K	putative 6-phosphogluconolactonase
rbsC	b3750	rbsC	ER2796_3863	GTC -> CTC	V305L	D-ribose transporter subunit
rep	b3778	rep	ER2796_3888	GCG -> GTC	G329V	DNA helicase and single-stranded DNA-dependent ATPase
rhlB	b3780	rhlB	ER2796_3890	CAC -> TAC	H153Y	ATP-dependent RNA helicase
wecA	b3784	wecA	ER2796_3895	ATG -> ATA	M256I	UDP-GlcNAc:undecaprenylphosphate GlcNAc-1-phosphate transferase
wzzE	b3785	wzzE	ER2796_3896	TCG -> CCG	S240P	Entobacterial Common Antigen (ECA) polysaccharide chain length modulation protein
yjC0	b4078	yjC0	ER2796_4217	GCC -> GTC	A54V	hypothetical protein
rnr	b4179	rnr	ER2796_4325	GAT -> AAT; GTG -> CTG	D225N; V354L	exoribonuclease R, RNase R
ppa	b4226	ppa	ER2796_4371	CCG -> TCG	P23S	inorganic pyrophosphatase
treR	b4241	treR	ER2796_4386	ACC -> ATC	T234I	DNA-binding transcriptional repressor
bdcA	b4249	bdcA	ER2796_4392	GGA -> AGA	G52R	c-di-GMP-binding biofilm dispersal mediator protein
idnO	b4266	idnO	ER2796_4409	GCG -> GAC	G85D	5-keto-D-glucuronate-5-reductase
yjjU	b4377	yjjU	ER2796_4486	CGG -> CAG	R126Q	putative phospholipase, patatin-like family
deoA	b4382	deoA	ER2796_4493	CCG -> TCG	P110S	thymidine phosphorylase
yjjJ	b4385	yjjJ	ER2796_4496	CTC -> TTC	L182F	putative protein kinase

creC	b4399	creC	ER2796_4511	CGC -> CCC	R77P	sensory histidine kinase in two-component regulatory system with CreB or PhoB, regulator of the CreBC regulon
yhdP	b4472	yhdP	ER2796_3330	CCG -> TCG	P1247S	conserved membrane protein, predicted transporter
insA	b4516	insA	ER2796_4627	TCA -> TTA	S87L	IS1 repressor TnpA