

Table S2. Genes required for growth or survival in the lung model detected by HITS

HI Locus	% TA Hit ^a	Survival Index ^b	COG ^c	Gene	Description
HI0761	74.6	0.000	M	mltC	murein transglycosylase C
HI0621.1	58.1	0.000	E	gmhB	D,D-heptose 1,7-bisphosphate phosphatase
HI0857	57.1	0.000	S	zapA	cell division associated protein
HI0187a	53.8	0.000	U	tatA	Sec-independent protein secretion pathway component TatA
HI0337	53.3	0.000	E	glnB	nitrogen regulatory protein P-II
HI0261	52.7	0.000	M	opsX	heptosyltransferase I
HI0854	51.1	0.000	P	-	putative heme iron utilization protein
HI1033	51.0	0.000	E	serB	phosphoserine phosphatase
HI1181	48.0	0.000	G	gmhA	phosphoheptose isomerase
HI0836	47.8	0.000	J	genX	lysyl-tRNA synthetase
HI1114	46.5	0.000	M	rfaD	ADP-L-glycero-D-mannoheptose-6-epimerase
HI0309	45.7	0.000	L	xerD	site-specific tyrosine recombinase XerD
HI0706	45.5	0.000	M	nlpD	lipoprotein
HI1086	44.1	0.000	Q	yrbE (mlaE)	ABC transporter permease
HI0032	43.5	0.000	M	pbp2	penicillin-binding protein 2
HI0847	42.9	0.000	S	-	hypothetical protein HI0847
HI0031	41.7	0.000	D	rodA	rod shape-determining protein
HI1105	40.0	0.000	M	rfaF	heptosyltransferase II
HI0812	68.4	0.011	M	galU	UDP-glucose pyrophosphorylase
HI0523	51.1	0.012	M	orfH	heptosyltransferase III
HI0428	46.4	0.012	O	dsbB	disulfide bond formation protein B
HI0942	45.5	0.014	L	recC	exodeoxyribonuclease V gamma chain
HI0551	55.3	0.014	T	apaH	diadenosine tetraphosphatase
HI0740	51.7	0.015	G	yhxB	phosphomannomutase (pgm)
HI0465	67.2	0.017	H	serA	D-3-phosphoglycerate dehydrogenase
HI0846	60.0	0.021	O	por	periplasmic oxidoreductase DsbA
HI1193	42.6	0.021	E	ilvE	branched-chain amino acid aminotransferase
HI0221	49.3	0.022	F	guaB	inositol-5-monophosphate dehydrogenase
HI0693	70.7	0.022	R	hel	lipoprotein E
HI0314	43.5	0.022	L	ruvC	Holliday junction resolvase
HI0141	43.5	0.023	G	nagB	glucosamine-6-phosphate deaminase
HI0334	54.5	0.024	T	relA	GTP pyrophosphokinase
HI0351	50.8	0.025	M	galE	UDP-glucose 4-epimerase
HI0770	65.3	0.028	D	ftsX	cell division protein FtsX
HI1087	54.3	0.028	Q	yrbF (mlaF)	ABC transporter ATPase
HI0290	60.8	0.029	P	-	putative cation-transporting ATPase
HI0286	59.6	0.031	E	alaT	aminotransferase AlaT
HI0039	57.9	0.033	M	mreD	rod shape-determining protein MreD
HI0138	51.9	0.033	L	rnhA	ribonuclease H
HI0312	57.4	0.038	L	ruvB	Holliday junction DNA helicase B
HI0361	43.8	0.040	P	yfeB	iron (chelated) transporter ATP-binding protein
HI0313	64.3	0.043	L	ruvA	Holliday junction DNA helicase motor protein
HI0464	67.7	0.048	G	rpiA	ribose-5-phosphate isomerase A
HI1617	57.1	0.050	E	aspC	aromatic amino acid aminotransferase
HI0066	48.1	0.051	M	amiB	N-acetylmuramoyl-L-alanine amidase
HI0769	67.7	0.052	D	ftsE	cell division ATP-binding protein
HI1702	56.0	0.060	E	metE	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
HI0896	60.6	0.063	D	ftsN	cell division protein
HI0639	54.4	0.065	F	purB	adenylosuccinate lyase
HI1633	68.9	0.066	F	purA	adenylosuccinate synthetase
HI0479	48.8	0.067	C	atpD	F0F1 ATP synthase subunit beta
HI0206	49.0	0.068	F	nadN	NAD nucleotidase
HI1380 ^d	45.7	0.070	P	pstB	phosphate transport system ATPase component

HI1290	40.7	0.070	E	tyrA	bifunctional chorismate mutase/prephenate dehydrogenase
HI0408	51.0	0.080	P	yebM	ABC transporter ATP-binding protein
HI0572	48.5	0.080	O	pgdX	peroxiredoxin hybrid Prx5
HI0461	62.3	0.081	S	-	hypothetical protein HI0461
HI0188	72.3	0.081	U	tatC	Sec-independent protein translocase protein TatC
HI0718	68.4	0.083	M	vacJ (mlaA)	lipoprotein
HI0676	48.8	0.083	L	xerC	site-specific tyrosine recombinase XerC
HI1145	59.4	0.084	E	pheA	chorismate mutase/prephenate dehydratase
HI0209	46.8	0.085	L	dam	DNA adenine methylase
HI1146	59.2	0.086	R	-	hypothetical protein HI1146
HI1277	45.1	0.086	D	mrp	putative ATPase
HI1494	40.0	0.091	V	-	N-acetylmuramoyl-L-alanine amidase
HI1615	61.9	0.096	F	purE	phosphoribosylaminoimidazole carboxylase catalytic subunit
HI1698	51.3	0.099	M	-	lipopolysaccharide biosynthesis protein
HI0407	54.5	0.100	P	-	hypothetical protein HI0407
HI0119	45.8	0.101	P	znuA	high-affinity zinc transporter periplasmic component
HI1263	46.7	0.102	E	metX	homoserine O-acetyltransferase
HI0329	59.5	0.102	E	-	hypothetical protein HI0329
HI0187b	75.0	0.102	U	tatB	sec-independent translocase
HI1248	63.5	0.103	R	-	hypothetical protein HI1248
HI1323	42.9	0.103	S	-	hypothetical protein HI1323
HI0887	63.9	0.105	F	purH	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
HI1249	71.4	0.106	R	-	hypothetical protein HI1249
HI0480	42.9	0.106	C	atpG	F0F1 ATP synthase subunit gamma
HI0086	68.4	0.109	E	metB	cystathionine gamma-synthase
HI0371	50.0	0.111	S	-	hypothetical protein HI0371
HI0669	40.0	0.111	C	mioC	flavodoxin
HI1389.1	50.7	0.112	E	trpC	bifunctional indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase
HI0330	43.9	0.114	M	oapA	opacity associated protein
HI1388	46.2	0.115	E	trpG	anthranilate synthase component II
HI1077	51.7	0.116	F	pyrG	CTP synthetase
HI1084	61.2	0.116	Q	yrbC (mlaC)	ABC transporter
HI1432	47.5	0.118	E	trpA	tryptophan synthase subunit alpha
HI1389	44.2	0.119	E	trpD	anthranilate phosphoribosyltransferase
HI0122	65.8	0.123	E	metC	cystathionine beta-lyase
HI0055	62.1	0.123	G	uxuA	mannonate dehydratase
HI0950	50.0	0.125	J	rpmG	50S ribosomal protein L33
HI1085	60.0	0.130	Q	yrbD (mlaD)	ABC transporter periplasmic protein
HI0416	52.4	0.133	H	thiD	phosphomethylpyrimidine kinase
HI1383m	54.2	0.141	P	pstS	phosphate ABC transporter phosphate-binding protein
HI1726	52.1	0.148	F	hemH	phosphoribosylaminoimidazole-succinocarboxamide synthase
HI1605	45.2	0.150	T	-	hypothetical protein HI1605
HI1431	47.9	0.151	E	trpB	tryptophan synthase subunit beta
HI1381	54.2	0.152	P	pstA	phosphate ABC transporter permease
HI0653	62.5	0.152	M	lgtF	LOS glycosyltransferase
HI1382	51.5	0.154	P	pstC	phosphate ABC transporter permease
HI1379	59.1	0.158	T	phoB	phosphate regulon transcriptional regulatory protein PhoB
HI0427	60.2	0.162	P	nhaB	sodium/proton antiporter
HI1713	50.0	0.167	G	ptsH	phosphocarrier protein HPr
HI0325	42.2	0.168	R	-	hypothetical protein HI0325
HI0462	52.1	0.172	O	lon	ATP-dependent proteinase
HI0756	62.0	0.177	D	-	hypothetical protein HI0756
HI0888	41.4	0.181	F	purD	phosphoribosylamine--glycine ligase
HI1207	72.7	0.184	F	purF	amidophosphoribosyltransferase
HI0518	62.5	0.185	F	deoD	purine nucleoside phosphorylase
HI0457	50.0	0.188	R	-	hypothetical protein HI0457

HI0752	60.2	0.188	F	purL	phosphoribosylformylglycinamide synthase
HI0062	52.9	0.190	T	dksA	dnaK suppressor protein
HI1387	49.4	0.195	E	trpE	anthranilate synthase component I
HI0415	41.4	0.213	H	thiM	hydroxyethylthiazole kinase
HI0980	76.2	0.217	K	fis	DNA-binding protein Fis
HI1171	54.5	0.225	E	trpG	para-aminobenzoate synthase component II
HI0176	42.6	0.231	J	rluD	23S rRNA pseudouridine synthase D
HI0038	47.1	0.235	M	mreC	rod shape-determining protein
HI1429	61.4	0.238	F	purM	phosphoribosylaminoimidazole synthetase
HI1658	59.3	0.241	R	-	hypothetical protein HI1658
HI1647	52.4	0.241	H	pdxS	pyridoxal biosynthesis lyase PdxS
HI1642	42.0	0.241	V	sapF	anti peptide resistance ABC transporter ATPase
HI0564	53.2	0.242	E	asnA	asparagine synthetase AsnA
HI1159m	48.7	0.243	O	ybbN	thioredoxin domain-containing protein
HI0535	56.0	0.245	O	ureH	urease accessory protein
HI0443	63.6	0.246	L	recR	recombination protein RecR
HI0011	68.2	0.256	L	hold	DNA polymerase III subunit psi
HI0571	57.8	0.256	K	oxyR	DNA-binding transcriptional regulator OxyR
HI0765	63.9	0.258	M	lpsA	LOS glycosyltransferase
HI0713	66.1	0.270	O	tig	trigger factor
HI0411	91.7	0.274	R	hfq	RNA-binding protein Hfq
HI0029	52.0	0.276	M	dacA	penicillin-binding protein 5
HI1004	59.8	0.279	O	ppiD	peptidyl-prolyl cis-trans isomerase
HI1103	42.5	0.282	E	cysK	cysteine synthetase
HI1191	65.5	0.285	R	-	hypothetical protein HI1191
HI0534	57.3	0.289	E	aspA	aspartate ammonia-lyase
HI1234	73.7	0.290	G	mgsA	methylglyoxal synthase

The set of *H. influenzae* genes listed in the table were determined by two criteria as depicted in Fig. 2. Genes were considered to be specifically required *in vivo* if they sustained insertions in at least 40% of the possible insertion sites in the internal 5-80% of the gene in the *in vitro* grown input library and the total number of sequencing reads mapping to insertion sites in each gene decreased at least 3.3-fold after *in vivo* passage relative to the input library.

^a Percentage of TA dinucleotides with detected insertions in the 5' 5 to 80% of each gene.

^b Survival index calculated as the fraction of total sequencing reads mapped to insertions in the output library relative to the input library.

^c Identifier for the Cluster of Orthologous Groups functional classification.

^d For HI1380, the gene *pstB* has been annotated as a containing an “artificial frameshift” and was not listed in the *H. influenzae* Rd KW20 (NC_000907.ptt) protein table.