

Supplementary material for

Identifying paths to ammonium production in *Vibrio natriegens* by characterizing nitrogenase regulation

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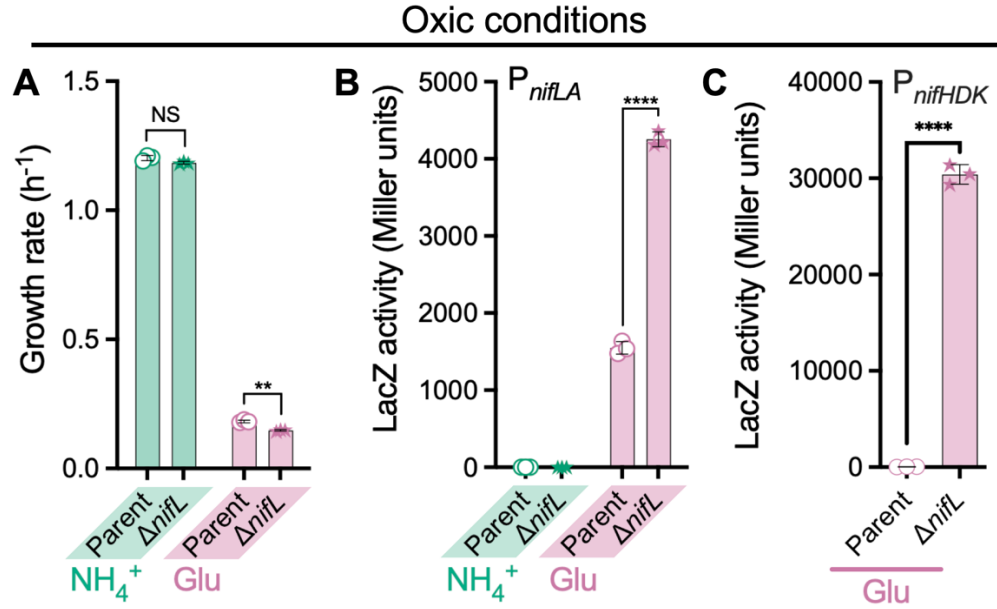


Fig S1. NifL prevents nitrogenase expression in response to O_2 . (A) *V. natriegens* growth rates in oxic minimal media with glucose and either NH_4^+ or glutamate as the nitrogen source. Parent, NWH003 ($\Delta dns::\text{Km}^R$); $\Delta nifL$, NWH037 ($\Delta dns::\text{Km}^R$). (B, C) A *lacZ* transcriptional reporter was used to compare expression from P_{nifLA} (B) or P_{nifHDK} (C). (A – C) Points, biological replicates; bars, mean; error bars, SD; $n=3$. Statistical differences were determined using an unpaired, two-tailed t tests; NS, non-significant; **, $P < 0.01$; ****, $P < 0.0001$.

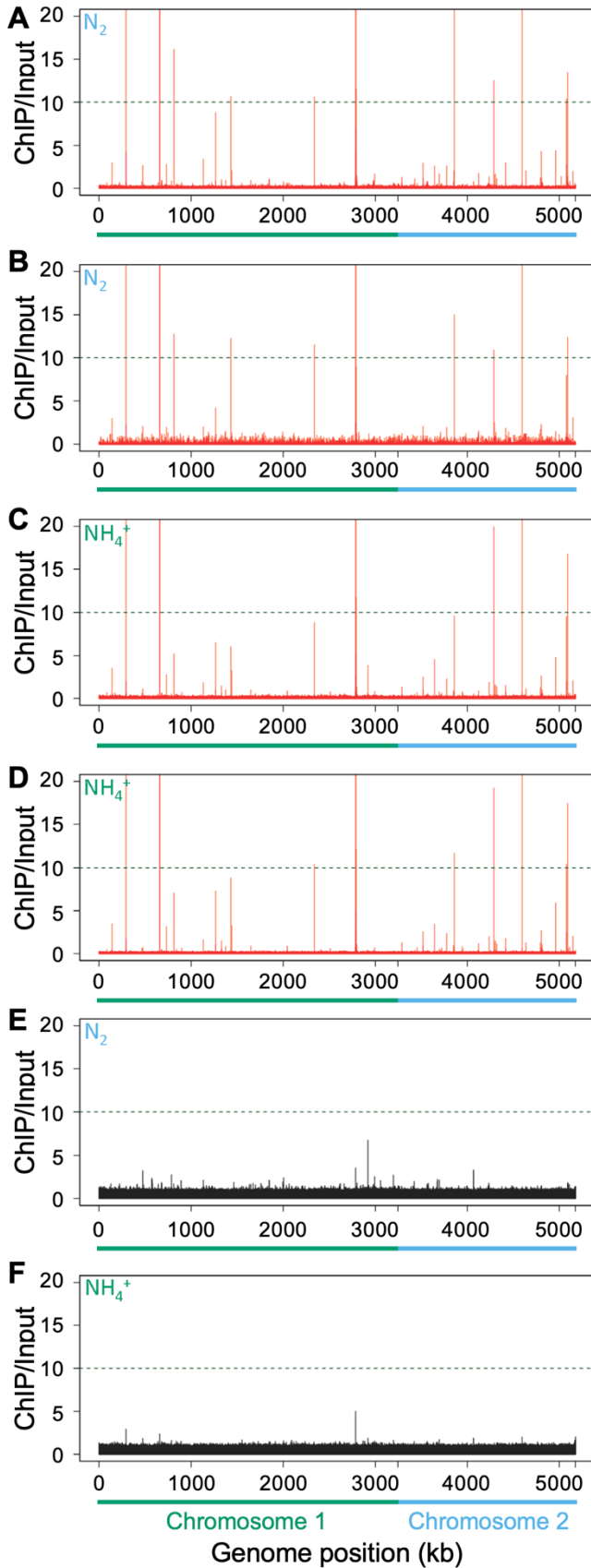


Fig S2. NtrC binds the same chromosomal sites when *V. natriegens* is grown with N_2 versus NH_4^+ . DNA binding of His-tagged NtrC was as determined by ChIP-seq using strain NWH071. Each plot with red bars shows a biological replicate with N_2 (**A, B**) or NH_4^+ (**C, D**). Plots with black bars are controls from strain NWH061 with untagged NtrC grown with N_2 (**E**) or NH_4^+ (**F**). Sequencing coverage values were determined for 1 kb bins across the genome for ChIP-enriched samples, and the coverage ratio relative to a whole-genome control is plotted on the Y-axis.

Table S1. *V. natriegens* genes expected to be involved in N₂ fixation^a

Gene (synonyms)	Description	Locus tag	Protein ID
Regulatory proteins			
<i>rpoN</i> (<i>ntrA</i> , <i>glnF</i>)	σ N/ σ 54	PN96_RS00495	WP_020334488
<i>ntrB</i> (<i>glnL</i> , <i>glnR</i>)	Nitrogen regulatory protein NR(II)	PN96_RS12940	WP_014230519
<i>ntrC</i> (<i>glnG</i> , <i>glnT</i>)	Nitrogen regulatory protein NR(I)	PN96_RS12945	WP_014230518
<i>glnD</i>	Bifunctional uridylyltransferase/uridylyl-removing enzyme	PN96_RS02290	WP_020335150
<i>glnB</i>	Nitrogen regulatory protein P-II	PN96_RS02395	WP_014232746
<i>glnK</i>	P-II family nitrogen regulator	PN96_RS01485	WP_005386570
<i>nifA</i>	<i>nif</i> -specific transcriptional activator	PN96_RS19610	WP_020333834
<i>nifL</i>	NifA anti-activator	PN96_RS19615	WP_020333835
Transporters			
<i>amtB1</i>	Ammonium transporter	PN96_RS01490	WP_014232922
<i>amtB2</i>	Ammonium transporter	PN96_RS05910	WP_020333273
<i>modA1</i>	Molybdate ABC transporter, substrate-binding protein	PN96_RS19520	WP_020333821
<i>modB1</i>	Molybdate ABC transporter, permease subunit	PN96_RS19525	WP_231119409
<i>modC1</i>	Molybdate ABC transporter, ATP-binding protein	PN96_RS19530	WP_020333823
<i>modA2</i>	Molybdate ABC transporter, substrate-binding protein	PN96_RS20775	WP_020334863
<i>modB2</i>	Molybdate ABC transporter, permease subunit	PN96_RS20780	WP_020334865
<i>modC2</i>	Molybdate ABC transporter, ATP-binding protein	PN96_RS20785	WP_024372990
Nitrogenase biosynthesis			
<i>nifH</i>	Nitrogenase Fe protein	PN96_RS19440	WP_020333808
<i>nifD</i>	Nitrogenase Mo-Fe protein, α -subunit	PN96_RS19445	WP_014234003
<i>nifK</i>	Nitrogenase Mo-Fe protein, β -subunit	PN96_RS19450	WP_014234004
<i>nifT</i>	Nitrogen fixation protein	PN96_RS19455	WP_020333810
<i>RS19460</i>	NifB/NifX family Mo-Fe cluster-binding protein	PN96_RS19460	WP_020333811
<i>RS19465</i>	Hypothetical protein	PN96_RS19465	WP_020333812
<i>nifE</i>	Nitrogenase Mo-Fe cofactor biosynthesis protein	PN96_RS19470	WP_020333813
<i>nifN</i>	Nitrogenase Mo-Fe cofactor biosynthesis protein	PN96_RS19475	WP_020333814
<i>RS19480</i>	NifB/NifX family Mo-Fe cluster-binding protein	PN96_RS19480	WP_014234009
<i>nifU</i>	Fe-S cluster assembly protein	PN96_RS19490	WP_020333815
<i>nifS</i>	Cysteine desulfurase	PN96_RS19495	WP_020333816
<i>nifV</i>	Homocitrate synthase	PN96_RS19500	WP_020333817
<i>nifW</i>	Nitrogenase-stabilizing/protective protein	PN96_RS19505	WP_020333818
<i>nifZ</i>	Nitrogen fixation protein	PN96_RS19510	WP_020333819
<i>nifM</i>	Peptidylprolyl isomerase	PN96_RS19515	WP_020333820
<i>nifQ</i>	Nitrogenase Mo-Fe cofactor biosynthesis protein	PN96_RS19595	WP_020333832
<i>nifB</i>	Nitrogenase Mo-Fe cofactor biosynthesis protein	PN96_RS19605	WP_236614651
Electron transfer			
<i>fdxB</i>	Ferredoxin III, <i>nif</i> -specific	PN96_RS19485	WP_014234010
<i>RS24415</i>	Putative ferredoxin	PN96_RS24415	WP_076633454
<i>nifF</i>	Flavodoxin	PN96_RS19655	WP_020333842
<i>rnfA</i>	RnfABCDGE type electron transport complex, subunit A	PN96_RS19620	WP_014234038
<i>rnfB</i>	RnfABCDGE type electron transport complex, subunit B	PN96_RS19625	WP_020333836
<i>rnfC</i>	RnfABCDGE type electron transport complex, subunit C	PN96_RS19630	WP_020333837
<i>rnfD</i>	RnfABCDGE type electron transport complex, subunit D	PN96_RS19635	WP_020333838
<i>rnfG</i>	RnfABCDGE type electron transport complex, subunit G	PN96_RS19640	WP_020333839
<i>rnfE</i>	RnfABCDGE type electron transport complex, subunit E	PN96_RS19645	WP_020333840
<i>rnfH</i>	RnfH family protein	PN96_RS19650	WP_020333841

^a*V. natriegens* proteins were identified based on existing annotations or from manual BLASTp hits that had > 25% identity across > 50% of the query sequence (i.e., NtrBC).

Table S2. Differentially expressed genes in nitrogen-starved *ΔntrBC* mutant versus the parent^a

Locus tag	Gene name	Description	Log ₂ fold-change
Downregulated genes in <i>ΔntrBC</i>			
PN96_RS12940	<i>ntrB (glnL)</i>	nitrogen regulation protein NR(II)	-18.90
PN96_RS12945	<i>ntrC (glnG)</i>	nitrogen regulation protein NR(I)	-18.61
PN96_RS19430	<i>cobA</i>	uroporphyrinogen-III C-methyltransferase	-16.54
PN96_RS17460	<i>eutB</i>	ethanolamine ammonia-lyase subunit EutB	-14.08
PN96_RS19625	<i>rnfB</i>	RnfABCDGE type electron transport complex subunit B	-13.63
PN96_RS17465	<i>eutC</i>	ethanolamine ammonia-lyase subunit EutC	-13.41
PN96_RS19440	<i>nifH</i>	nitrogenase Fe protein	-13.30
PN96_RS19635	<i>rnfD</i>	RnfABCDGE type electron transport complex subunit D	-12.94
PN96_RS19620	<i>rnfA</i>	RnfABCDGE type electron transport complex subunit A	-12.85
PN96_RS19630	<i>rnfC</i>	RnfABCDGE type electron transport complex subunit C	-12.47
PN96_RS19445	<i>nifD</i>	nitrogenase Fe-Mo protein α-chain	-12.44
PN96_RS19490	<i>nifU</i>	Fe-S cluster assembly protein NifU	-12.37
PN96_RS19450	<i>nifK</i>	nitrogenase Fe-Mo protein β-subunit	-12.03
PN96_RS19495	<i>nifS</i>	cysteine desulfurase NifS	-11.93
PN96_RS16310		PLP-dependent aminotransferase family protein	-11.93
PN96_RS17800		nucleobase:cation symporter-2 family protein	-11.85
PN96_RS16080		AraC family transcriptional regulator	-11.76
PN96_RS19640	<i>rnfG</i>	RnfABCDGE type electron transport complex subunit G	-11.52
PN96_RS19455	<i>nifT</i>	putative nitrogen fixation protein NifT	-11.44
PN96_RS19500	<i>nifV</i>	homocitrate synthase	-11.29
PN96_RS19505	<i>nifW</i>	nitrogenase-stabilizing/protective protein NifW	-11.28
PN96_RS06645		helix-turn-helix domain-containing protein	-11.16
PN96_RS19510	<i>nifZ</i>	nitrogen fixation protein NifZ	-10.91
PN96_RS19515	<i>nifM</i>	peptidylprolyl isomerase	-10.88
PN96_RS20310		transporter substrate-binding protein	-10.87
PN96_RS19645	<i>rnfE</i>	RnfABCDGE type electron transport complex subunit E	-10.74
PN96_RS19605	<i>nifB</i>	nitrogenase cofactor biosynthesis protein NifB	-10.67
PN96_RS19615	<i>nifL</i>	nitrogen fixation negative regulator NifL	-10.66
PN96_RS19595	<i>nifQ</i>	nitrogen fixation protein NifQ	-10.61
PN96_RS17470	<i>eat</i>	ethanolamine permease	-10.61
PN96_RS19460		NifB/NifX family Fe-Mo cluster-binding protein	-10.61
PN96_RS24200		hypothetical protein	-10.56
PN96_RS19470	<i>nifE</i>	nitrogenase Fe-Mo cofactor biosynthesis protein NifE	-10.56
PN96_RS19650	<i>rnfH</i>	RnfH family protein	-10.53
PN96_RS19520	<i>modA1</i>	molybdate ABC transporter substrate-binding protein	-10.51
PN96_RS03150		PLP-dependent aspartate aminotransferase family protein	-10.44
PN96_RS19525	<i>modB1</i>	molybdate ABC transporter permease subunit	-10.42
PN96_RS19465		hypothetical protein	-10.40
PN96_RS19655	<i>nifF</i>	flavodoxin	-10.25
PN96_RS19530	<i>modC1</i>	molybdenum ABC transporter ATP-binding protein	-10.02

PN96_RS19475	<i>nifN</i>	nitrogenase Fe-Mo cofactor biosynthesis protein NifN	-10.01
PN96_RS20315		amino acid ABC transporter permease	-9.95
PN96_RS01485	<i>glnK</i>	PII family nitrogen regulator	-9.92
PN96_RS19610	<i>nifA</i>	nif-specific transcriptional activator NifA	-9.89
PN96_RS19480		NifB/NifX family Fe-Mo cluster-binding protein	-9.83
PN96_RS19485	<i>fdxB</i>	ferredoxin III, nif-specific	-9.60
PN96_RS17830	<i>xdhA</i>	xanthine dehydrogenase small subunit	-9.58
PN96_RS03145		transporter substrate-binding domain-containing protein	-9.58
PN96_RS17455		helix-turn-helix domain-containing protein	-9.49
PN96_RS03475		hypothetical protein	-9.36
PN96_RS19750		AraC family transcriptional regulator	-9.26
PN96_RS20320		amino acid ABC transporter permease	-9.22
PN96_RS17815		2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline decarboxylase	-9.03
PN96_RS17825	<i>xdhB</i>	xanthine dehydrogenase molybdopterin binding subunit	-8.98
PN96_RS17820	<i>xdhC</i>	xanthine dehydrogenase accessory protein XdhC	-8.86
PN96_RS17810	<i>uraH</i>	hydroxyisourate hydrolase	-8.86
PN96_RS03140		amino acid ABC transporter permease	-8.79
PN96_RS01490	<i>amtB1</i>	ammonium transporter	-8.77
PN96_RS03920		hypothetical protein	-8.71
PN96_RS17805		urate hydroxylase PuuD	-8.60
PN96_RS20325		amino acid ABC transporter ATP-binding protein	-8.38
PN96_RS10880		ABC transporter substrate-binding protein	-8.13
PN96_RS03795		circularly permuted type 2 ATP-grasp protein	-7.92
PN96_RS10885		ABC transporter ATP-binding protein	-7.91
PN96_RS18585		muconate/chloromuconate family cycloisomerase	-7.75
PN96_RS21135	<i>glgC</i>	glucose-1-phosphate adenylyltransferase	-7.70
PN96_RS03135		amino acid ABC transporter ATP-binding protein	-7.56
PN96_RS03790		α-E domain-containing protein	-7.56
PN96_RS16315		TSUP family transporter	-7.48
PN96_RS19425	<i>nirB</i>	nitrite reductase large subunit NirB	-7.46
PN96_RS10890		ABC transporter permease	-7.43
PN96_RS03785		transglutaminase family protein	-7.26
PN96_RS04685		glycosyltransferase	-7.25
PN96_RS23215	<i>gabT</i>	4-aminobutyrate--2-oxoglutarate transaminase	-7.23
PN96_RS06690		AraC family transcriptional regulator	-7.20
PN96_RS16070		RidA family protein	-7.19
PN96_RS19740		hypothetical protein	-7.18
PN96_RS19420	<i>nirD</i>	nitrite reductase small subunit NirD	-7.15
PN96_RS23225		TRAP transporter substrate-binding protein	-6.99
PN96_RS10875		amidohydrolase family protein	-6.97
PN96_RS23370		AraC family ligand binding domain-containing protein	-6.93
PN96_RS10895		2-oxoglutarate and Fe-dependent oxygenase domain-containing protein	-6.89

PN96_RS23230		TRAP transporter small permease	-6.72
PN96_RS19745	<i>putA</i>	bifunctional proline dehydrogenase/L-glutamate gamma-semialdehyde dehydrogenase PutA	-6.67
PN96_RS19035	<i>guaD</i>	guanine deaminase	-6.53
PN96_RS06405		NCS1 family nucleobase:cation symporter-1	-6.47
PN96_RS19415		NarK family nitrate/nitrite MFS transporter	-6.34
PN96_RS05590		amino acid ABC transporter substrate-binding protein	-6.29
PN96_RS20305		MurR/RpiR family transcriptional regulator	-6.26
PN96_RS19435		nitrate reductase	-6.23
PN96_RS16320		SDR family NAD(P)-dependent oxidoreductase	-6.20
PN96_RS04760		STAS domain-containing protein	-6.20
PN96_RS17835		TetR/AcrR family transcriptional regulator	-6.20
PN96_RS23210		PLP-dependent aminotransferase family protein	-6.18
PN96_RS22690		GlxA family transcriptional regulator	-6.17
PN96_RS04680		glycosyltransferase family 2 protein	-6.11
PN96_RS23220		NAD-dependent succinate-semialdehyde dehydrogenase	-5.98
PN96_RS22045		phytanoyl-CoA dioxygenase family protein	-5.86
PN96_RS23235		TRAP transporter large permease subunit	-5.84
PN96_RS05585		amino acid ABC transporter permease	-5.80
PN96_RS11180	<i>ung</i>	uracil-DNA glycosylase	-5.72
PN96_RS04700		acyltransferase	-5.70
PN96_RS05580		amino acid ABC transporter permease	-5.63
PN96_RS07685		hybrid-cluster NAD(P)-dependent oxidoreductase	-5.62
PN96_RS04755		OmpA family protein	-5.58
PN96_RS19730	<i>aspA</i>	aspartate ammonia-lyase	-5.55
PN96_RS22005		DUF4440 domain-containing protein	-5.50
PN96_RS24300		hypothetical protein	-5.49
PN96_RS19735	<i>putP</i>	sodium/proline symporter PutP	-5.46
PN96_RS05575		amino acid ABC transporter ATP-binding protein	-5.40
PN96_RS04715		glycosyltransferase family 4 protein	-5.30
PN96_RS12935		DUF4124 domain-containing protein	-5.16
PN96_RS23265	<i>dgcN</i>	N-acetyltransferase DgcN	-5.15
PN96_RS07680	<i>hcp</i>	hydroxylamine reductase	-5.09
PN96_RS21995		hypothetical protein	-5.08
PN96_RS21975		FAD-dependent oxidoreductase	-5.07
PN96_RS15975		aromatic amino acid transporter	-5.06
PN96_RS22010		SDR family oxidoreductase	-4.91
PN96_RS15970	<i>tnaA</i>	tryptophanase	-4.90
PN96_RS23270	<i>dgcA</i>	N-acetyl-D-Glu racemase DgcA	-4.90
PN96_RS19410		bifunctional protein-serine/threonine kinase/phosphatase	-4.86
PN96_RS22015		S-(hydroxymethyl)glutathione dehydrogenase/class III alcohol dehydrogenase	-4.84
PN96_RS04710		oligosaccharide flippase family protein	-4.73
PN96_RS23275		D-amino-acid transaminase	-4.73

PN96_RS01495		Fe³⁺ ABC transporter substrate-binding protein	-4.72
PN96_RS04720		glycosyltransferase family 4 protein	-4.72
PN96_RS04675		sugar transferase	-4.71
PN96_RS21970		flavodoxin domain-containing protein	-4.67
PN96_RS05910	<i>amtB2</i>	ammonium transporter	-4.67
PN96_RS06400		amidase	-4.60
PN96_RS06410		GntR family transcriptional regulator	-4.48
PN96_RS13155		Flp family type IVb pilin	-4.41
PN96_RS23060		helix-turn-helix domain-containing protein	-4.28
PN96_RS19360		choline ABC transporter substrate-binding protein	-4.25
PN96_RS20515	<i>dld</i>	D-lactate dehydrogenase	-4.15
PN96_RS22030		cytochrome P450	-4.14
PN96_RS06395		gamma-glutamyltransferase family protein	-4.14
PN96_RS12950		GGDEF and EAL domain-containing protein	-4.07
PN96_RS15200		polysaccharide biosynthesis/export family protein	-4.07
PN96_RS06415		aspartate/glutamate racemase family protein	-4.04
PN96_RS17225		ABC transporter substrate-binding protein	-4.01
PN96_RS23280		CPBP family intramembrane glutamic endopeptidase	-4.00
PN96_RS23260	<i>alr</i>	alanine racemase	-3.94
PN96_RS15550		L-lactate permease	-3.93
PN96_RS15230		glycosyltransferase	-3.92
PN96_RS15195		outer membrane β -barrel protein	-3.89
PN96_RS21480		D-(-)-3-hydroxybutyrate oligomer hydrolase	-3.89
PN96_RS06420	<i>puuE</i>	allantoinase PuuE	-3.86
PN96_RS03275		OmpA family protein	-3.79
PN96_RS15210		putative capsular polysaccharide synthesis family protein	-3.79
PN96_RS06390		alanine--glyoxylate aminotransferase family protein	-3.76
PN96_RS15555	<i>lldD</i>	FMN-dependent L-lactate dehydrogenase LldD	-3.72
PN96_RS06695		EthD domain-containing protein	-3.67
PN96_RS04705		O-antigen ligase family protein	-3.67
PN96_RS04695		glycosyltransferase	-3.67
PN96_RS03060		hypothetical protein	-3.66
PN96_RS17420		hypothetical protein	-3.64
PN96_RS06385		allantoate amidohydrolase	-3.62
PN96_RS15220		O-antigen ligase family protein	-3.56
PN96_RS19355	<i>betA</i>	choline dehydrogenase	-3.54
PN96_RS15205		polysaccharide biosynthesis tyrosine autokinase	-3.53
PN96_RS21310		glycosyl transferase family protein	-3.52
PN96_RS15225		putative capsular polysaccharide synthesis family protein	-3.52
PN96_RS16085		hypothetical protein	-3.50
PN96_RS15190		undecaprenyl-phosphate glucose phosphotransferase	-3.50
PN96_RS00590	<i>arcA</i>	arginine deiminase	-3.47
PN96_RS06380		DUF5718 family protein	-3.46

PN96_RS07915		hypothetical protein	-3.46
PN96_RS17845		hypothetical protein	-3.46
PN96_RS15215		glycosyltransferase	-3.45
PN96_RS17415		LruC domain-containing protein	-3.45
PN96_RS15235		oligosaccharide flippase family protein	-3.44
PN96_RS06700		MFS transporter	-3.43
PN96_RS06275	<i>ureA</i>	urease subunit gamma	-3.41
PN96_RS22025		TetR/AcrR family transcriptional regulator	-3.38
PN96_RS17230		branched-chain amino acid ABC transporter permease	-3.38
PN96_RS22585		hypothetical protein	-3.34
PN96_RS17065		porin	-3.32
PN96_RS22685		dipeptidase	-3.26
PN96_RS16325		ABC transporter ATP-binding protein	-3.23
PN96_RS06245		HupE/UreJ family protein	-3.23
PN96_RS17525		DUF134 domain-containing protein	-3.22
PN96_RS06255		urease accessory UreF family protein	-3.22
PN96_RS17530		NifB/NifX family Fe-Mo cluster-binding protein	-3.21
PN96_RS05970		DUF3305 domain-containing protein	-3.19
PN96_RS16355	<i>ahpC</i>	alkyl hydroperoxide reductase subunit C	-3.19
PN96_RS05955		molecular chaperone	-3.15
PN96_RS06265	<i>ureC</i>	urease α -subunit	-3.14
PN96_RS05105		EamA family transporter	-3.14
PN96_RS06250	<i>ureG</i>	urease accessory protein UreG	-3.13
PN96_RS17535	<i>nqrM</i>	(Na ⁺)-NQR maturation NqrM	-3.13
PN96_RS06285	<i>urtE</i>	urea ABC transporter ATP-binding subunit UrtE	-3.13
PN96_RS06260	<i>ureE</i>	urease accessory protein UreE	-3.12
PN96_RS05965		DUF3306 domain-containing protein	-3.12
PN96_RS22995		glutaredoxin	-3.11
PN96_RS09125		TRAP transporter substrate-binding protein	-3.09
PN96_RS06280		urease accessory protein UreD	-3.07
PN96_RS06270		urease β -subunit	-3.06
PN96_RS17540		hypothetical protein	-3.06
PN96_RS15240		VanZ family protein	-3.06
PN96_RS09120		TRAP transporter small permease	-3.03
PN96_RS13150		prepilin peptidase	-2.99
PN96_RS06290	<i>urtD</i>	urea ABC transporter ATP-binding protein UrtD	-2.98
PN96_RS06540	<i>hcaC</i>	3-phenylpropionate/cinnamic acid dioxygenase ferredoxin subunit	-2.98
PN96_RS09670	<i>flgK</i>	flagellar hook-associated protein FlgK	-2.98
PN96_RS12720		energy transducer TonB	-2.95
PN96_RS12695		TonB-dependent receptor	-2.87
PN96_RS16395		1-pyrroline-5-carboxylate dehydrogenase	-2.87
PN96_RS09750		FlgO family outer membrane protein	-2.86
PN96_RS05960		4Fe-4S dicluster domain-containing protein	-2.86
PN96_RS05950		transcriptional initiation protein Tat	-2.85

PN96_RS09675	<i>flgJ</i>	flagellar assembly peptidoglycan hydrolase FlgJ	-2.84
PN96_RS12930	<i>glnA</i>	glutamate--ammonia ligase	-2.84
PN96_RS02605		flagellar protein FliT	-2.83
PN96_RS18640		Lrp/AsnC family transcriptional regulator	-2.82
PN96_RS04690		chain-length determining protein	-2.82
PN96_RS15470		hypothetical protein	-2.80
PN96_RS13415	<i>fadB</i>	fatty acid oxidation complex α -subunit FadB	-2.79
PN96_RS12705		MotA/TolQ/ExbB proton channel family protein	-2.78
PN96_RS09660		flagellin	-2.78
PN96_RS12715		biopolymer transporter ExbD	-2.77
PN96_RS16390	<i>putP</i>	sodium/proline symporter PutP	-2.76
PN96_RS12710		MotA/TolQ/ExbB proton channel family protein	-2.76
PN96_RS06295	<i>urtC</i>	urea ABC transporter permease subunit UrtC	-2.75
PN96_RS16360	<i>ahpF</i>	alkyl hydroperoxide reductase subunit F	-2.74
PN96_RS12700		DUF3450 domain-containing protein	-2.74
PN96_RS19795		transporter substrate-binding domain-containing protein	-2.71
PN96_RS13420	<i>fadA</i>	acetyl-CoA C-acyltransferase FadA	-2.71
PN96_RS19725	<i>mgo</i>	malate dehydrogenase (quinone)	-2.70
PN96_RS16400	<i>putA</i>	bifunctional proline dehydrogenase/L-glutamate gamma-semialdehyde dehydrogenase PutA	-2.69
PN96_RS24345		ABC transporter ATP-binding protein	-2.67
PN96_RS09745	<i>flgP</i>	flagellar assembly lipoprotein FlgP	-2.66
PN96_RS06300	<i>urtB</i>	urea ABC transporter permease subunit UrtB	-2.64
PN96_RS06375		chromate transporter	-2.63
PN96_RS04750		SLBB domain-containing protein	-2.62
PN96_RS08175		hypothetical protein	-2.62
PN96_RS12725		hypothetical protein	-2.61
PN96_RS17240		amidohydrolase family protein	-2.58
PN96_RS20765		hypothetical protein	-2.58
PN96_RS09680		flagellar basal body P-ring protein FlgI	-2.58
PN96_RS19800		response regulator	-2.57
PN96_RS06535	<i>hcaF</i>	phenylpropionate/cinnamic acid dioxygenase β -subunit	-2.56
PN96_RS19850		MFS transporter	-2.56
PN96_RS19370	<i>choV</i>	choline ABC transporter ATP-binding protein	-2.54
PN96_RS18285		SDR family oxidoreductase	-2.53
PN96_RS24340		branched-chain amino acid ABC transporter ATP-binding protein/permease	-2.52
PN96_RS05940	<i>fdh3B</i>	formate dehydrogenase FDH3 β -subunit	-2.52
PN96_RS05945		formate dehydrogenase α -subunit	-2.52
PN96_RS03780		circularly permuted type 2 ATP-grasp protein	-2.50
PN96_RS07415		cysteine desulfurase-like protein	-2.50
PN96_RS07420		2OG-Fe dioxygenase family protein	-2.47
PN96_RS20430		TRAP transporter small permease subunit	-2.44
PN96_RS19790		ATP-binding protein	-2.44

PN96_RS06305	<i>urtA</i>	urea ABC transporter substrate-binding protein	-2.43
PN96_RS22900		OmpA family protein	-2.42
PN96_RS02600	<i>fliD</i>	flagellar filament capping protein FliD	-2.42
PN96_RS05935		formate dehydrogenase subunit gamma	-2.40
PN96_RS19365	<i>choW</i>	choline ABC transporter permease subunit	-2.39
PN96_RS13145	<i>cpaB</i>	Flp pilus assembly protein CpaB	-2.38
PN96_RS17935		SDR family oxidoreductase	-2.35
PN96_RS03995		calcium-binding protein	-2.35
PN96_RS09685	<i>flgH</i>	flagellar basal body L-ring protein FlgH	-2.34
PN96_RS09690	<i>flgG</i>	flagellar basal-body rod protein FlgG	-2.33
PN96_RS23255		sodium:proton antiporter	-2.32
PN96_RS15025		redoxin family protein	-2.26
PN96_RS22035		APC family permease	-2.22
PN96_RS20665		DUF1800 domain-containing protein	-2.21
PN96_RS24035		IS3 family transposase - programmed frameshift	-2.21
PN96_RS02610	<i>fliS</i>	flagellar export chaperone FliS	-2.20
PN96_RS18290		acetyl-CoA C-acetyltransferase	-2.20
PN96_RS11990	<i>cysD</i>	sulfate adenylyltransferase subunit CysD	-2.19
PN96_RS16300	<i>tauA</i>	taurine ABC transporter substrate-binding protein	-2.18
PN96_RS15350		type I secretion system permease/ATPase	-2.17
PN96_RS09695		flagellar basal body rod protein FlgF	-2.17
PN96_RS22120	<i>fdrA</i>	acyl-CoA synthetase FdrA	-2.17
PN96_RS18345		hypothetical protein	-2.15
PN96_RS17220		membrane protein	-2.13
PN96_RS18645		ornithine cyclodeaminase	-2.12
PN96_RS23040		methyl-accepting chemotaxis protein	-2.12
PN96_RS00935		LysE/ArgO family amino acid transporter	-2.12
PN96_RS09755		flagellar assembly protein FlgT	-2.11
PN96_RS15355		calcium-binding protein	-2.11
PN96_RS03915		septation protein A	-2.09
PN96_RS22785		4a-hydroxytetrahydrobiopterin dehydratase	-2.07
PN96_RS09665	<i>flgL</i>	flagellar hook-associated protein FlgL	-2.06
PN96_RS16290		ABC transporter permease subunit	-2.06
PN96_RS11985	<i>cysN</i>	sulfate adenylyltransferase subunit CysN	-2.04
PN96_RS05385		aldehyde dehydrogenase family protein	-2.04
PN96_RS02595	<i>flaG</i>	flagellar protein FlaG	-2.04
PN96_RS19540		ExeM/NucH family extracellular endonuclease	-2.02
PN96_RS15360		LuxR C-terminal-related transcriptional regulator	-2.00
Upregulated genes in $\Delta ntrBC$			
PN96_RS11665		lysophospholipid acyltransferase family protein	7.73
PN96_RS13625	<i>punC</i>	purine nucleoside transporter PunC	7.38
PN96_RS09210		isochorismatase family protein	7.15
PN96_RS15300		aromatic ring-hydroxylating dioxygenase α -subunit	7.03
PN96_RS16160		SulP family inorganic anion transporter	6.96

PN96_RS07115		ATP-binding protein	6.57
PN96_RS08155		tRNA-Ser	2.80
PN96_RS08160		L-alanine exporter AlaE	2.76
PN96_RS17795		ribosome recycling factor family protein	2.20
PN96_RS14570	<i>frdD</i>	fumarate reductase subunit FrdD	2.15
PN96_RS11245	<i>gltB</i>	glutamate synthase large subunit	2.01
PN96_RS14580		succinate dehydrogenase/fumarate reductase Fe-S subunit	2.00

^aBold, genes directly regulated by NtrC as determined by ChIP-seq

Table S3. Differentially expressed genes in nitrogen-starved *ΔnifA* mutant versus the parent

Locus tag	Gene	Description	Log ₂ fold-change
Downregulated genes in <i>ΔnifA</i>			
PN96_RS19445	<i>nifD</i>	nitrogenase Fe-Mo protein α-subunit	-10.69
PN96_RS19440	<i>nifH</i>	nitrogenase Fe protein	-10.51
PN96_RS19450	<i>nifK</i>	nitrogenase Fe-Mo protein β-subunit	-10.34
PN96_RS19455	<i>nifT</i>	putative nitrogen fixation protein NifT	-10.22
PN96_RS19490	<i>nifU</i>	Fe-S cluster assembly protein NifU	-10.16
PN96_RS19620	<i>rnfA</i>	RnfABCDGE type electron transport complex subunit A	-10.06
PN96_RS19495	<i>nifS</i>	cysteine desulfurase NifS	-10.05
PN96_RS19625	<i>rnfB</i>	RnfABCDGE type electron transport complex subunit B	-9.76
PN96_RS19500	<i>nifV</i>	homocitrate synthase	-9.69
PN96_RS19460		NifB/NifX family Fe-Mo cluster-binding protein	-9.69
PN96_RS19465		hypothetical protein	-9.62
PN96_RS19630	<i>rnfC</i>	RnfABCDGE type electron transport complex subunit C	-9.56
PN96_RS19505	<i>nifW</i>	nitrogenase-stabilizing/protective protein NifW	-9.48
PN96_RS19510	<i>nifZ</i>	nitrogen fixation protein NifZ	-9.30
PN96_RS19515	<i>nifM</i>	peptidylprolyl isomerase	-9.19
PN96_RS19520	<i>modA1</i>	molybdate ABC transporter substrate-binding protein	-9.14
PN96_RS24200		hypothetical protein	-9.06
PN96_RS19635	<i>rnfD</i>	RnfABCDGE type electron transport complex subunit D	-9.01
PN96_RS19470	<i>nifE</i>	nitrogenase Fe-Mo cofactor biosynthesis protein NifE	-8.98
PN96_RS19525	<i>modB1</i>	molybdate ABC transporter permease subunit	-8.78
PN96_RS19640	<i>rnfG</i>	RnfABCDGE type electron transport complex subunit G	-8.70
PN96_RS19530	<i>modC1</i>	molybdenum ABC transporter ATP-binding protein	-8.68
PN96_RS19480		NifB/NifX family Fe-Mo cluster-binding protein	-8.62
PN96_RS19475	<i>nifN</i>	nitrogenase Fe-Mo cofactor biosynthesis protein NifN	-8.58
PN96_RS19485	<i>fdxB</i>	ferredoxin III, nif-specific	-8.51
PN96_RS19645	<i>rnfE</i>	RnfABCDGE type electron transport complex subunit E	-8.50
PN96_RS19650	<i>rnfH</i>	RnfH family protein	-8.09
PN96_RS19655	<i>nifF</i>	flavodoxin	-7.99
PN96_RS19610	<i>nifA</i>	nif-specific transcriptional activator NifA	-5.13
PN96_RS07685		hybrid-cluster NAD(P)-dependent oxidoreductase	-4.82
PN96_RS07680	<i>hcp</i>	hydroxylamine reductase	-4.30
PN96_RS19605	<i>nifB</i>	nitrogenase cofactor biosynthesis protein NifB	-2.83
PN96_RS16355	<i>ahpC</i>	alkyl hydroperoxide reductase subunit C	-2.79
PN96_RS19595	<i>nifQ</i>	nitrogen fixation protein NifQ	-2.76
PN96_RS17525		DUF134 domain-containing protein	-2.74
PN96_RS17530		NifB/NifX family Fe-Mo cluster-binding protein	-2.72
PN96_RS19550		DMT family transporter	-2.71
PN96_RS15025		redoxin family protein	-2.66
PN96_RS17535	<i>nqrM</i>	(Na ⁺)-NQR maturation NqrM	-2.58
PN96_RS19660		hypothetical protein	-2.45

PN96_RS17540		hypothetical protein	-2.44
PN96_RS16360	<i>ahpF</i>	alkyl hydroperoxide reductase subunit F	-2.40
PN96_RS24035		IS3 family transposase - programmed frameshift	-2.26
PN96_RS04695		glycosyltransferase	-2.23
PN96_RS19540		ExeM/NucH family extracellular endonuclease	-2.00

Table S4. Bacterial strains

Strain/ designation	Derived from	Relevant genotype ^a	Comments	Source
TND1964/ wild-type	ATCC 14048	pMMB- <i>tfoX</i> (Vc)		[1]
NWH003/ Parent	TND1964	$\Delta dns::Km^R$		This study
NWH004/ Parent	TND1964	$\Delta dns::Sp^R$		This study
NWH005	TND1964	$\Delta ntrBC$ $\Delta dns::Sp^R$	Intermediate used to make NWH014, 043, and 064	This study
NWH006	TND1964	$\Delta dns::Km^R-lacZ$	Intermediate used to make NWH010 and 017	This study
NWH010/ Parent	NWH006	$\Delta dns::Sp^R-P_{nifLA}-lacZ$	Parent with P_{nifLA} -LacZ reporter	This study
NWH014/ $\Delta ntrBC$	NWH005	$\Delta ntrBC$, $\Delta dns::Km^R$		This study
NWH015/ $\Delta ntrBC$	NWH014	$\Delta ntrBC$ $\Delta dns::Sp^R-P_{nifLA}-lacZ$	$\Delta ntrBC$ mutant with P_{nifLA} -LacZ reporter	This study
NWH017	NWH006	$\Delta dns::Sp^R-lacZ$	Promoterless LacZ control	This study
NWH036/ $\Delta nifA$	TND1964	$\Delta nifA$, $\Delta dns::Km^R$		This study
NWH037/ $\Delta nifL$	TND1964	$\Delta nifL$, $\Delta dns::Km^R$		This study
NWH143	NWH006	$\Delta dns::Sp^R-P_{nifLA}-nifL-lacZ$	Intermediate used to make NWH144	This study
NWH144/ $\Delta nifL$, $P_{nifLA}-nifL$	NWH037	$\Delta nifL$, $\Delta dns::Sp^R-P_{nifLA}-nifL-lacZ$	$\Delta nifL$ mutant with P_{nifLA} -NifL-LacZ reporter and NifL complementation	This study
NWH145/ $\Delta nifL$, $P_{nifLA}-nifL$	NWH037	$\Delta nifL$, $\Delta dns::Sp^R-P_{nifLA}-nifL$	$\Delta nifL$ mutant with P_{nifLA} -NifL complementation	This study
NWH038/ Δrnf	TND1964	Δrnf , $\Delta dns::Km^R$	<i>rnf</i> operon deletion (<i>RS19650-19620</i>)	This study
NWH039/ $\Delta nifF$	TND1964	$\Delta nifF$, $\Delta dns::Km^R$		This study
NWH042/ $\Delta glnB$	TND1964	$\Delta glnB$, $\Delta dns::Km^R$		This study
NWH043/ $\Delta ntrBC\Delta nifA$	NWH005	$\Delta ntrBC$, $\Delta nifA$, $\Delta dns::Km^R$		This study
NWH044/ Parent	NWH006	$\Delta dns::Sp^R-P_{nifHDK}-lacZ$	Parent with P_{nifHDK} -LacZ reporter	This study
NWH047/ $\Delta glnK$	TND1964	$\Delta glnK$, $\Delta dns::Km^R$		This study
NWH050/ ΔPII	NWH042	$\Delta glnB$, $\Delta glnK$, $\Delta dns::Sp^R$		This study
NWH060	TND1964	$\Delta dns::Km^R-P_{ntrBC}-ntrBC$	Intermediate used to make NWH064	This study
NWH061	NWH064	$\Delta ntrBC$, $\Delta dns::Sp^R-P_{glnA}-ntrBC$	$\Delta ntrBC$ complemented. Untagged ChIP- seq control	This study
NWH064	NWH005	$\Delta ntrBC$, $\Delta dns::Km^R-P_{ntrBC}-ntrBC$	Intermediate used to make NWH061	This study
NWH066	TND1964	$\Delta dns::Sp^R-P_{glnA}-ntrB-ntrC::His6x$	Intermediate used to make NWH071	This study
NWH071	NWH014	$\Delta ntrBC$, $\Delta dns::Sp^R-P_{glnA}-ntrB-ntrC::His6x$	$\Delta ntrBC$ complemented with WT <i>ntrB</i> and a C-terminal His6x-tagged <i>ntrC</i> for ChIP-seq	This study
NWH090/ $\Delta nifA$	NWH036	$\Delta nifA$ $\Delta dns::Sp^R-P_{nifLA}-lacZ$	$\Delta nifA$ mutant with P_{nifLA} -LacZ reporter	This study
NWH105/ parent	TND1964	$\Delta dns::Sp^R-P_{tet}-nifA-P_{lacI}-tetR$	Parent with aTc-inducible <i>nifA</i>	This study
NWH106/ $\Delta ntrBC$	NWH014	$\Delta ntrBC$, $\Delta dns::Sp^R-P_{tet}-nifA-P_{lacI}-tetR$	$\Delta ntrBC$ mutant with aTc-inducible <i>nifA</i>	This study
NWH108/ $\Delta ntrBC\Delta nifA$	NWH043	$\Delta ntrBC$, $\Delta nifA$ $\Delta dns::Sp^R-P_{tet}-nifA-P_{lacI}-tetR$	$\Delta ntrBC\Delta nifA$ mutant with aTc-inducible <i>nifA</i>	This study
NWH111 $\Delta PII\Delta ntrBC$	NWH042	$\Delta glnB$, $\Delta glnK$, $\Delta ntrBC$, $\Delta dns::Sp^R$		This study

NWH112 $\Delta\Ptext{II}\Delta nifA$	NWH042	$\Delta glnB, \Delta glnK, \Delta nifA,$ $\Delta dns::Sp^R$		This study
NWH126/ $\Delta ntrBC\Delta nifA$	NWH043	$\Delta ntrBC, \Delta nifA,$ $\Delta dns::Sp^R-P_{nifLA}-lacZ$	$\Delta ntrBC\Delta nifA$ mutant with P_{nifLA} -LacZ reporter	This study
NWH127/ $\Delta ntrBC\Delta nifA, P_{tet}-nifA$	NWH043	$\Delta ntrBC, \Delta nifA,$ $\Delta dns::Sp^R-P_{nifLA}-lacZ-$ $P_{tet}-nifA-P_{lacI}-tetR$	$\Delta ntrBC\Delta nifA$ mutant with P_{nifLA} -LacZ reporter and aTc-inducible $nifA$	This study

^aAll strains retain the pMMB-*tfoX* (Vc) plasmid following cloning, but the plasmid was omitted from other rows for brevity. All strains are thus resistance to carbenicillin due to the resistance cassette on the plasmid.

Table S5. Primers

Primer	Sequence (5'→3') ^a	Description ^b
NH014	CTCTGCACCACTACCGTC	F; $\Delta dns::XXX$ screening
NH015	CGAATACCGATGTCGCTGC	R; $\Delta dns::XXX$ screening
NH022	CGAGGTGAAGATCATTCATTTC	F; universal primer; upstream for $\Delta dns::XXX$
NH025	CTTAGTGATTGGGTCACTCATTGG	R; universal primer; downstream for $\Delta dns::XXX$
NH028	CTATGGACACGGGTAATCATACC	R; $\Delta dns::Sp^R$ internal for screening
NH031	CTCGATGTCGTTTCGAGTCC	F; upstream for $\Delta ntrBC$; SOE
NH032	aatcaaagtgctagtaactcctCACATTATGTCCTTGTTGCG	R; upstream for $\Delta ntrBC$
NH033	ttaacgcaaacaaggacataatgtgAAGGAGTTGTACTGACACTTTG	F; downstream for $\Delta ntrBC$
NH034	GATCACCCAGTACCGCTAC	R; downstream for $\Delta ntrBC$; SOE
NH035	GAGGGACTCACACTCTTTTACG	F; $\Delta ntrBC$ screening
NH036	CCGACAGTTGCTTATCACTG	R; $\Delta ntrBC$ screening
NH041	ctgcaggtcgacggatccccggaatCACACAGGAAACAGCTATGACCATG	F; pHRP309 <i>lacZ</i> + RBS in $\Delta dns::Km^R-lacZ$
NH042	tttaactatgaaatacctgttctctCTGTGCGACCTGCTTGATCG	R; pHRP309 <i>lacZ</i> + RBS in $\Delta dns::Km^R-lacZ$
NH043	cgattgtcgcgattggtgaggattaTGAGGCTGGAGCTGCTTC	F; For Km^R in $\Delta dns::Km^R-lacZ$
NH044	catggtcatagctgttctgtgtgATTCCGGGGATCCGTCGAC	R; For Km^R in $\Delta dns::Km^R-lacZ$
NH045	aacttcgaagcagctccagcctacaTAATCCTCACCAATCGCGAC	R; upstream of <i>dns</i> for $\Delta dns::Km^R-lacZ$
NH046	ggccgcgatcaagcaggtcgacagAGAGAACAGGTATTCATAGTTAAAGTC	F; downstream for of <i>dns</i> for $\Delta dns::Km^R-lacZ$
NH049	CGCTTGCCTTTTCCCTTG	F; $\Delta dns::Sp^R$ internal for screening
NH053	CAGACCATTTTCAATCCGCAC	R; universal SOE for $P_{xxx}-lacZ$ fusions; 5' in <i>lacZ</i>
NH054	tggcatagctatgcaccacctcgCACTTTATGCTTCCGACCTG	R; upstream for $\Delta dns::Sp^R-P_{nifLA}-lacZ$, Sp^R-P_{nifLA} junction
NH055	ggctgcaggtcggaagcataaagtgCGAAGGTGGTGCATAGCG	F; for $\Delta dns::Sp^R-P_{nifLA}-lacZ$, Sp^R-P_{nifLA} junction; $\Delta nifL$ screening
NH056	catggtcatagctgttctgtgtgCGTTTGGCGATATAAGACCGTC	R; for $\Delta dns::Sp^R-P_{nifLA}-lacZ$, $P_{nifLA}-lacZ$ junction
NH057	atcgacggtcttatatcgccaacgCACACAGGAAACAGCTATGACCATG	F; for $\Delta dns::Sp^R-P_{nifLA}-lacZ$, <i>lacZ</i> downstream arm
NH251	CGTTTGGCGATATAAGACCGTC	R; upstream for $\Delta dns::Sp^R-P_{nifLA}-nifL-lacZ$ and $\Delta dns::Sp^R-P_{nifLA}-nifL$, $P_{nifLA}-nifL$ junction
NH252	GACGGTCTTATATCGCCAAAC	F; for $\Delta dns::Sp^R-P_{nifLA}-nifL-lacZ$ and $\Delta dns::Sp^R-P_{nifLA}-nifL$, $P_{nifLA}-nifL$ junction
NH253	catggtcatagctgttctgtgtgGCTGTCTAGATCGTTACG	R; for $\Delta dns::Sp^R-P_{nifLA}-nifL-lacZ$, <i>nifL-lacZ</i> junction
NH254	gatttagagcgtcagttattggcggGTGAAAGTGGGTCCTGATAAGG	F; downstream for $\Delta dns::Sp^R-P_{nifLA}-nifL$, <i>nifL::Δdns</i> junction
NH058	GGCTGAATATCGACGGTTTTCC	F; $\Delta dns::lacZ$ internal screening
NH064	catggtcatagctgttctgtgtgCACTTTATGCTTCCGACCTG	R; to replace Km^R with Sp^R in $\Delta dns::Km^R-lacZ$
NH065	ggctgcaggtcggaagcataaagtgCACACAGGAAACAGCTATGACCATG	F; to replace Km^R with Sp^R in $\Delta dns::Km^R-lacZ$
NH105	CAGCTCTGGAATGTCTTG	F; upstream for Δrnf ; SOE
NH106	gtcgacggatccccggaatCAATCCTTCGGACATAACAG	R; upstream for Δrnf

NH107	agaagcagctccagcctacaGATTAATTGTCACCTTTGCG	F; downstream for Δrnf
NH108	CGAGAACTAATCCAAACTTC	R; downstream Δrnf ; SOE
NH109	CGATATAAGACCGTCGATG	F; Δrnf screening
NH110	CGGTTTATCCGCAACCTC	R; Δrnf screening
NH111	GGTGCATGTGGATATGTTTG	F; upstream for $\Delta nifF$; SOE
NH112	gtcgacggatccccggaatGCTCATAATTAGCTCCTTG	R; upstream for $\Delta nifF$
NH113	agaagcagctccagcctacaGAGCTATGAATAAAATAAGCTATTAAC	F; downstream for $\Delta nifF$
NH114	GACATTATTATTACGAGTCGC	R; downstream for $\Delta nifF$; SOE
NH115	GATTAATTGTCACCTTTGCG	F; $\Delta nifF$ screening
NH116	CCTATTGTTACCATTC	R; $\Delta nifF$ screening
NH117	agaagcagctccagcctacaAACGATCTCAGACAGCCGCC	F; downstream for $\Delta nifL$
NH118	CCGCCAATAACTGACGCTC	R; $\Delta nifL$ screening; $P_{nifLA-lacZ}::P_{tet-nifA}$ screening
NH119	CCAAGTGATACATTAATAGACG	F; upstream for $\Delta glnB$; SOE
NH120	gtcgacggatccccggaatCTTCATGTTCCCTTAAG	R; upstream for $\Delta glnB$
NH121	agaagcagctccagcctacaGCGATTAAACATTTCACTC	F; downstream for $\Delta glnB$
NH122	CTCCTCAGGAACAAGAAG	R; downstream for $\Delta glnB$; SOE
NH123	CGTCACTGCTGTCTGATG	F; $\Delta glnB$ screening
NH124	CTCGATGCATTGAATATCTTCAG	R; $\Delta glnB$ screening
NH125	gcatgcagagaagaaccctcCACTTTATGCTTCCGACCTG	R; upstream for $\Delta dns::Sp^R-P_{nifHDK-lacZ}$, Sp^R-P_{nifHDK} junction
NH126	caggtcggaagcataaagtGAGGGTTCTTCTCTGCATG	F; for $\Delta dns::Sp^R-P_{nifHDK-lacZ}$, Sp^R-P_{nifHDK} junction
NH127	tcatagctgttctgtgtgTAATTTAGACTCATGACCGTTG	R; for $\Delta dns::Sp^R-P_{nifHDK-lacZ}$, $P_{nifHDK-lacZ}$ junction
NH128	acggtcatgagtctaaattaCACACAGGAAACAGCTATGACCATG	F; for $\Delta dns::Sp^R-P_{nifHDK-lacZ}$, $lacZ$ downstream arm
NH129	GATGAGCTTAAGACGCTC	F; upstream for $\Delta glnK$; SOE
NH130	gtcgacggatccccggaatCATTAAATTCTCTCCGAAGC	R; upstream for $\Delta glnK$
NH131	agaagcagctccagcctacaGCACTTTAAAGTATTCAAGGG	F; downstream for $\Delta glnK$
NH132	CGAATATCCGGGAACCG	R; downstream for $glnK$; SOE
NH133	CTGTGAAAGCACCGCTAG	F; $\Delta glnK$ screening
NH134	CGCCGTTATCAACGTACAT	R; $\Delta glnK$ screening
NH136	ctgcaggtcgacggatccccggaatGACCGTAAACGGGGCAAAG	F; for $\Delta dns::Km^R-P_{ntrBC-ntrBC}$, Km^R-P_{ntrBC}
NH137	tttaactatgaaatacctgttctctGTCTAATCACAAAAGGGTGGTG	R; for $\Delta dns::Km^R-P_{ntrBC-ntrBC}$, $ntrBC::\Delta dns$ junction
NH138	gcgcaccaccctttgtgattagacAGAGAACAGGTATTTATAGTTAAAGTC	F; for $\Delta dns::Km^R-P_{ntrBC-ntrBC}$, $ntrBC::\Delta dns$ downstream arm
NH139	ctgcaggtcgacggatccccggaatCTCTCTGGCGACATTTGC	F; for replacing Km^R-P_{ntrBC} with Sp^R-P_{glnA} in $\Delta dns::Km^R-P_{ntrBC-ntrBC}$; Sp^R-P_{glnA} junction
NH140	tccacattatgtccttgttgcgttAAATAAGCGGTAAAAGCTCG	R; for replacing Km^R-P_{ntrBC} with Sp^R-P_{glnA} in $\Delta dns::Km^R-P_{ntrBC-ntrBC}$; $P_{glnA-ntrB}$ junction
NH141	taaatcgagcttttaccgcttattAACGCAAACAAGGACATAATG	F; for replacing Km^R-P_{ntrBC} with Sp^R-P_{glnA} in $\Delta dns::Km^R-P_{ntrBC-ntrBC}$; $P_{glnA-ntrB}$ junction
NH142	GCTAAACTTGGTCAGATCG	R; for replacing Km^R-P_{ntrBC} with Sp^R-P_{glnA} in $\Delta dns::Km^R-P_{ntrBC-ntrBC}$; downstream arm
NH147	tggtggtgtgaacctgaaccGTACAACTCCTTTAATTTCTGGTG	R; for $\Delta dns::Sp^R-P_{glnA-ntrB-ntrC}::His6x$; $ntrC-His6$ junction

NH148	GGTTCAGGTTACACCACCACCACCAC	F; linker + Hisx6 tag for C-terminal NtrC fusion
NH149	GTGGTGGTGGTGGTGGTGAACCTGAACC	R; linker + Hisx6 tag for C-terminal NtrC fusion
NH150	cacaccaccaccaccaccacTGACACTTTGATTAATAATACAGCATAGC	F; for $\Delta dns::Sp^R-P_{glnA}-ntrB-ntrC::His6x$; linker +Hisx6 downstream arm
NH222	CTAGTATTTCCCCTCTTTCTCTAG	R; upstream for $P_{tet}-nifA-P_{lacI}-tetR$; $P_{tet}-nifA$ junction
NH223	CTCGGTACCAAATTCAGAAAAAG	F; downstream for $P_{tet}-nifA-P_{lacI}-tetR$; - $nifA-P_{lacI}$ junction
NH224	actagagaagagggaataactagATGATAGAAGATCATTCTCTTTAGATTAGAG	F; for $P_{tet}-nifA-P_{lacI}-tetR$; $P_{tet}-nifA$ junction
NH225	ctcttttctggaatttggtaccgagTCAGATTGTTTCATCTCGATATT	R; for $P_{tet}-nifA-P_{lacI}-tetR$; $nifA-P_{lacI}$ junction
NH235	tggtgtgtctcaaatctctgatgCCTGTTCTCTCTGTCGCAC	R; upstream for $\Delta dns::Sp^R-P_{nifLA}-lacZ-P_{tet}-nifA-P_{lacI}-tetR$; $nifA-P_{lacI}$; $lacZ-P_{tet}$ junction
NH236	CATCAGAGATTTTGAGACACAACC	F; downstream for $\Delta dns::Sp^R-P_{nifLA}-lacZ-P_{tet}-nifA-P_{lacI}-tetR$; $nifA-P_{lacI}$; $lacZ-P_{tet}$ junction
NH237	CGGTCGCTACCATTACCAG	F; $P_{nifLA}-lacZ::P_{tet}-nifA$ internal screening
SB001	GGACATGATTCACTCCTAAACCG	F; upstream for $\Delta nifA$; SOE
JL005	gtcgacggatccccggaatCCGCCAATAACTGACGCTC	R; upstream for $\Delta nifA$
JL006	gaagcagctccagcctacaCAAGGCTGCTCAATATGACGC	F; downstream for $\Delta nifA$
JL007	GAGATAAGTGACGCAAGCCG	R; downstream for $\Delta nifA$; SOE
JL008	AACTGTTGCTCCTTCTCAGCG	F; upstream for $\Delta nifL$; SOE
JL011	TGGTTTGTCTGCTCTGTCGC	R; downstream for $\Delta nifL$; SOE
JL016	gtcgacggatccccggaatGTAATGCTGATGGCGAC	R; upstream for $\Delta nifL$
JL049	TCAGATTGTTTCATCTCGATATT	R; $\Delta nifA$ screening
JL050	ATGATAGAAGATCATTCTCTTTTAGATTAGAG	F; $\Delta nifA$ screening
ABD123	ATTCCGGGGATCCGTCGAC	F; universal FRT for Sp^R or Km^R
ABD124	TGTAGGCTGGAGCTGCTTC	R; universal FRT for Sp^R or Km^R
miniFRT-F	ATTCCGGGGATCCGTCGACCTGCAGTTCAGAAGCAGCTCCAGCCTACA	F; Universal linker for deletion constructs
miniFRT-R	TGTAGGCTGGAGCTGCTTCTGAACTGCAGGTCGACGGATCCCCGGAAT	R; Universal linker for deletion constructs

^aLowercase indicates overlapping regions for SOE PCR.

^bF, forward; R, reverse

References for supplemental material

1. Dalia, T. N., Hayes, C. A., Stolyar, S., Marx, C. J., McKinlay, J. B. & Dalia, A. B. (2017). Multiplex genome editing by natural transformation (MuGENT) for synthetic biology in *Vibrio natriegens*. ACS Synth Biol. **6**, 1650-1655.
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