A.

VapB-1

```
        -8           2          12          22          32          42
MASMTGGQQM GRDPNSSSML TKVFQSGNSQ AVRIPMDFRF DVDTVEIFRK ENGDVVLRPV

        52          62          72
SKKTDDFLAL FEGFDETFIQ ALEARDDLPP QERENL
```

B.

VapC-1

```
        10          20          30          40          50          60
MIYMLDTNII IYLMKNRPKI IAERVSQLLP NDRLVMSFIT YAELIKGAFG SQNYEQSIRA

        70          80          90         100         110         120
IELLTERVNV LYPNEQICLH YGKWANTLKK QGRPIGNNDL WIACHALSLN AVLITHNVKE

       130         140
FQRITDLQWQ DWTKLEHHHH HH
```

Figure S1. Primary sequence of the VapBC-1 complex used for crystallization screening. A) VapB-1 and B) VapC-1. The underlined residues are from the pET24b cloning vector.

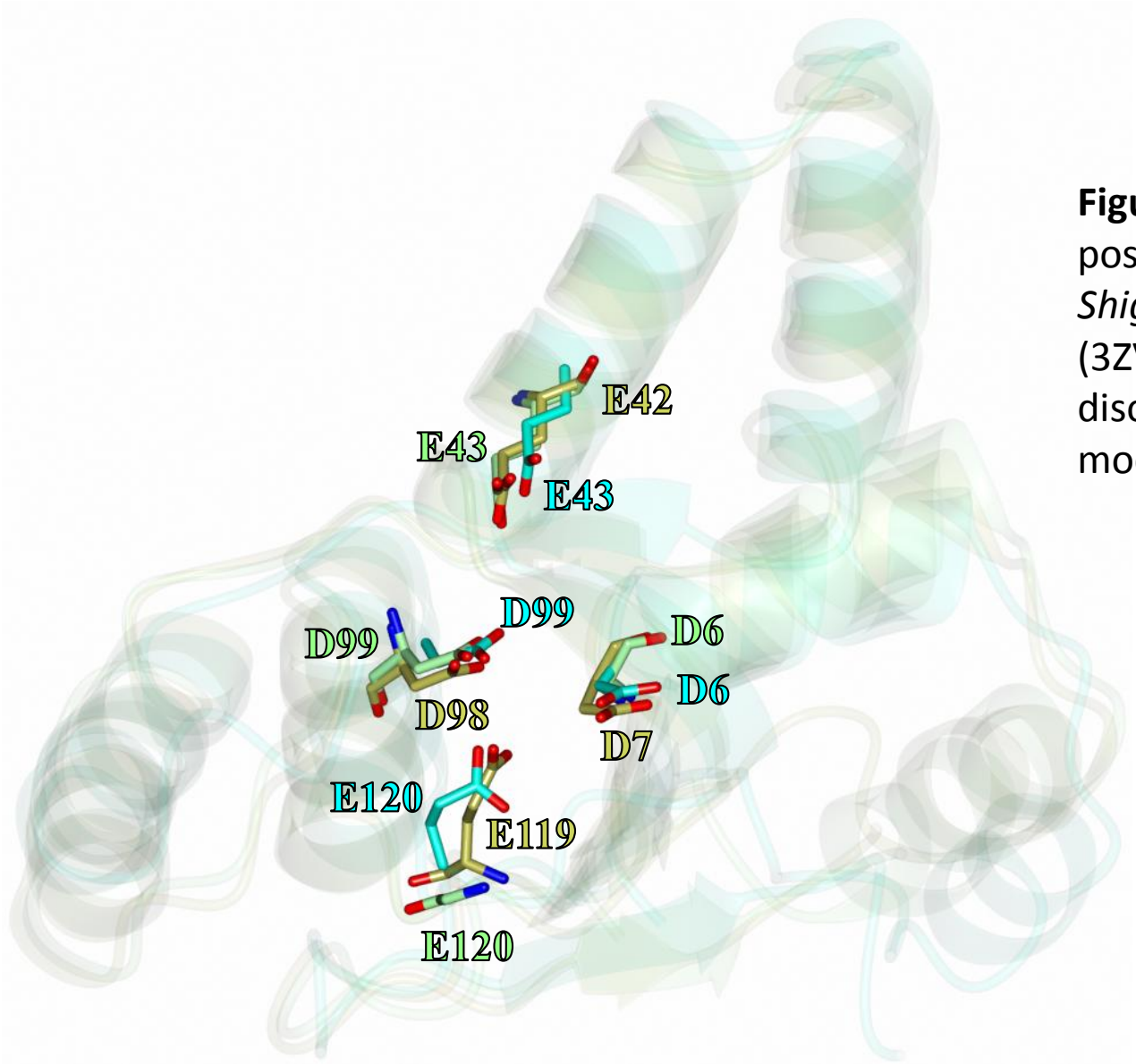


Figure S2. Comparison of the active site residue positions of VapC-1 (subunit B, cyan) with *Shigella flexneri* (3TND, gold) and *Rickettsia felis* (3ZVK, green). Residue E120 in 3ZVK is disordered and the backbone atoms only were modeled.

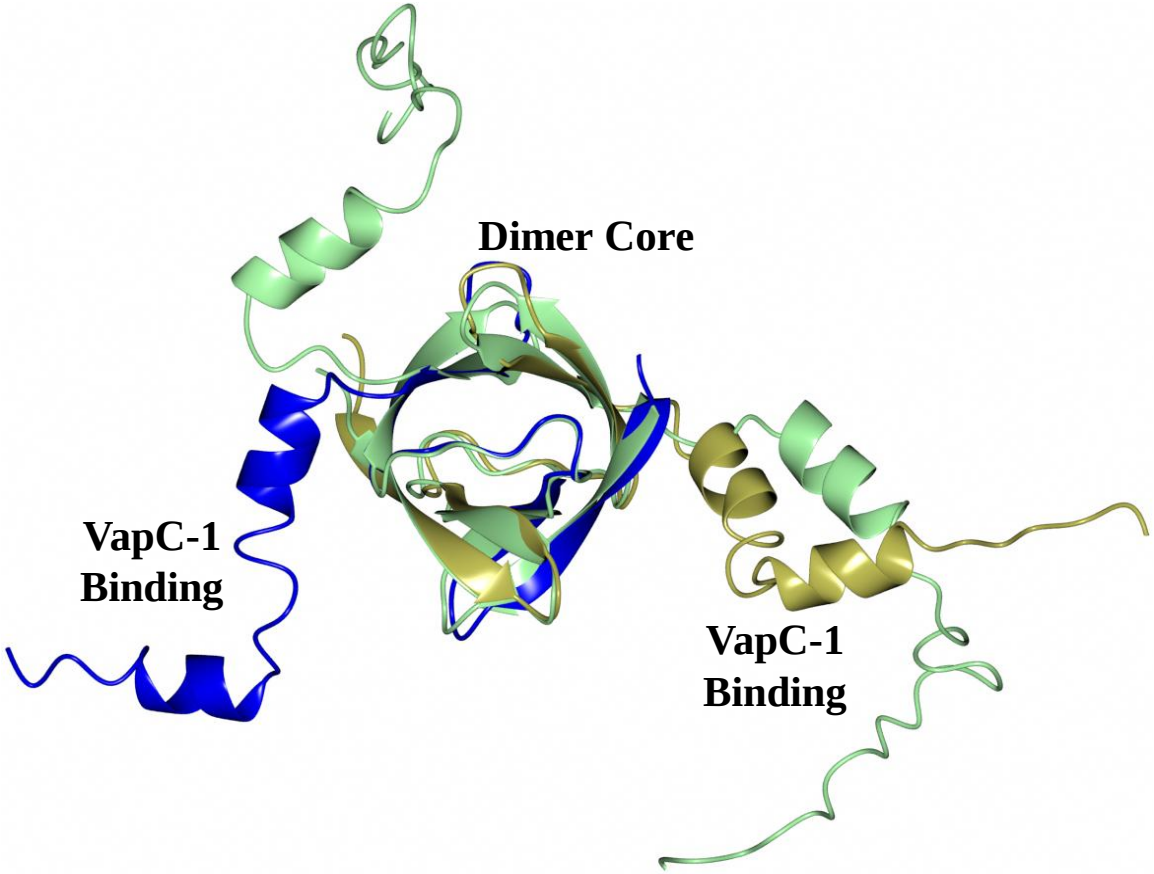


Figure S3. Comparison of the *H. influenzae* VapB-1 dimer (gold/blue) with the VapB dimer of *R. felis* (green) from the VapBC structure in complex with DNA (PDB 3ZVK).

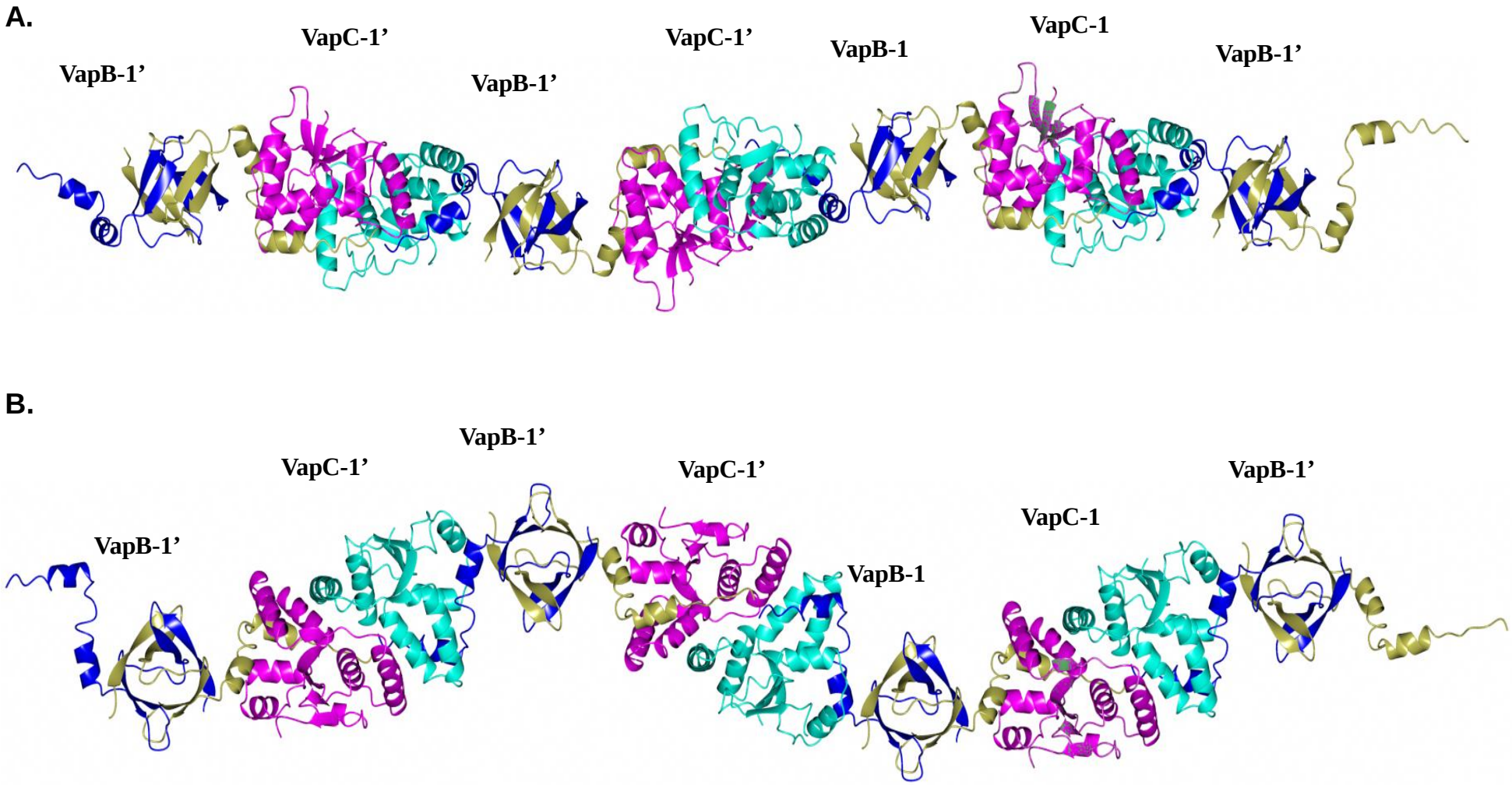
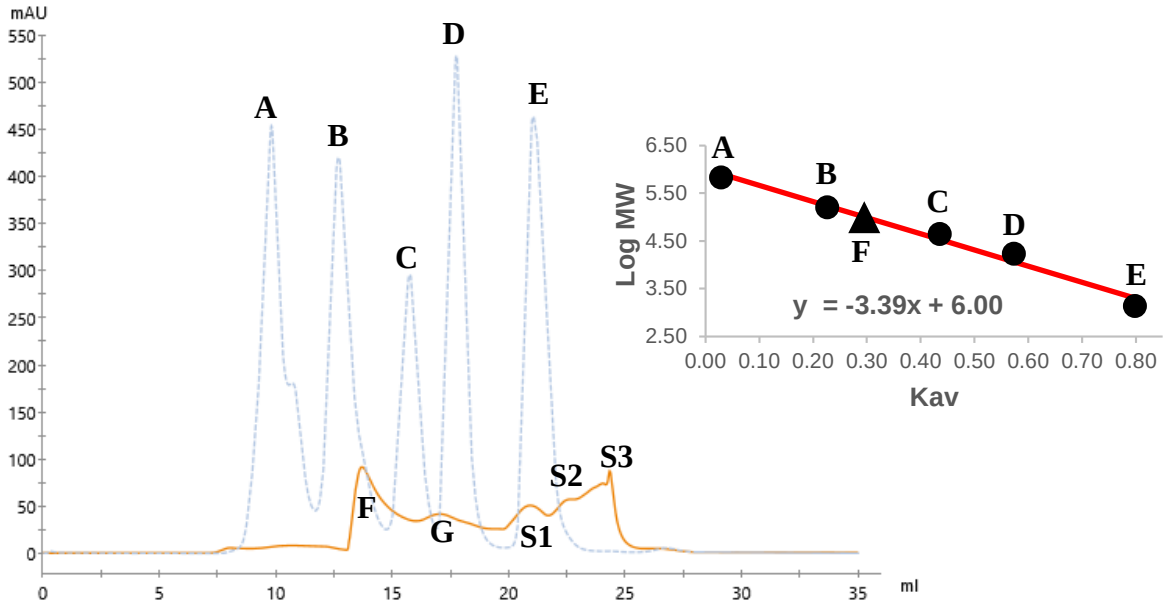


Figure S4. Crystal packing of VapBC-1 with VapB-1 chains C/D (gold/blue) and VapC-1 chains A/B (magenta/cyan). The VapBC-1 dimers form a linear chain that propagates in a spiral (2_1 screw) along the crystallographic *c*-axis. VapB-1' and VapC-1' molecules are those related by crystallographic symmetry. A) Viewed along the crystallographic *a*-axis and B) *b*-axis.

A.



Peak	Protein Standard	Molecular Weight (Da)	Elution Volume (mL)	Kav	Log MW
A	Thyroglobulin	670,000	9.82	0.03	5.90
B	gamma globulin	158,000	12.7	0.23	5.24
C	Ovalbumin	44,000	15.75	0.44	4.53
D	Myoglobin	17,000	17.76	0.57	4.06
E	Vitamin B12	1,350	21.05	0.80	3.30
F	VapBC	101,000	13.7	0.30	5.00
G	VapBC	17,000	17.06	0.52	4.22

B.

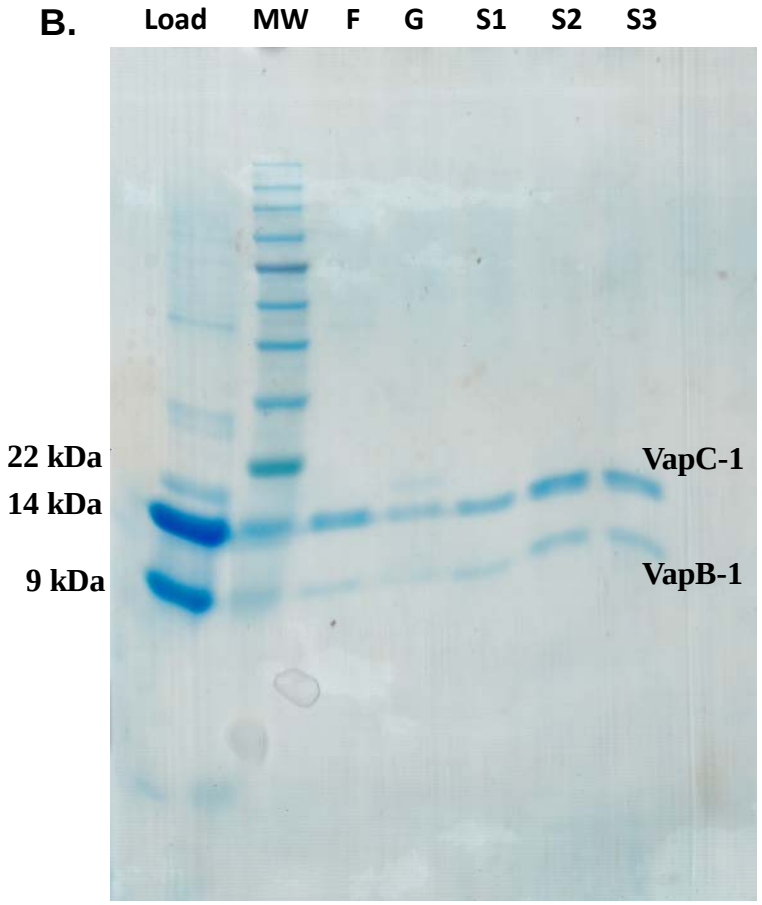


Figure S5. Size exclusion chromatography (Superdex 200) of the coexpressed VapBC-1 complex. A) Chromatogram showing the elution volumes of the calibration standards (blue dashed line) superimposed with the VapBC sample (orange solid line). The calibration plot (Log MW vs Kav) is shown as an inset on the chromatogram and the data points for each standard is represented as a black circle and the best-fit line determined by linear regression is indicated by the red line. The estimated molecular weight of the first VapBC-1 fraction (peak F), based on the column calibration, is indicated by the triangle. Elution volumes and molecular weights are provided in the table. Kav = (Ve-Vo)/(Vc-Vo) which accounts for the sample elution volume (Ve), void volume of the column (Vo) and the geometric column volume (Vc). The additional peaks that contained VapBC-1, which are outside of the column calibration, are noted as peaks S1-S3. B) SDS-PAGE analysis of the VapBC-1 elution fractions. Samples were analyzed for peak fractions F, G and S1-S3.

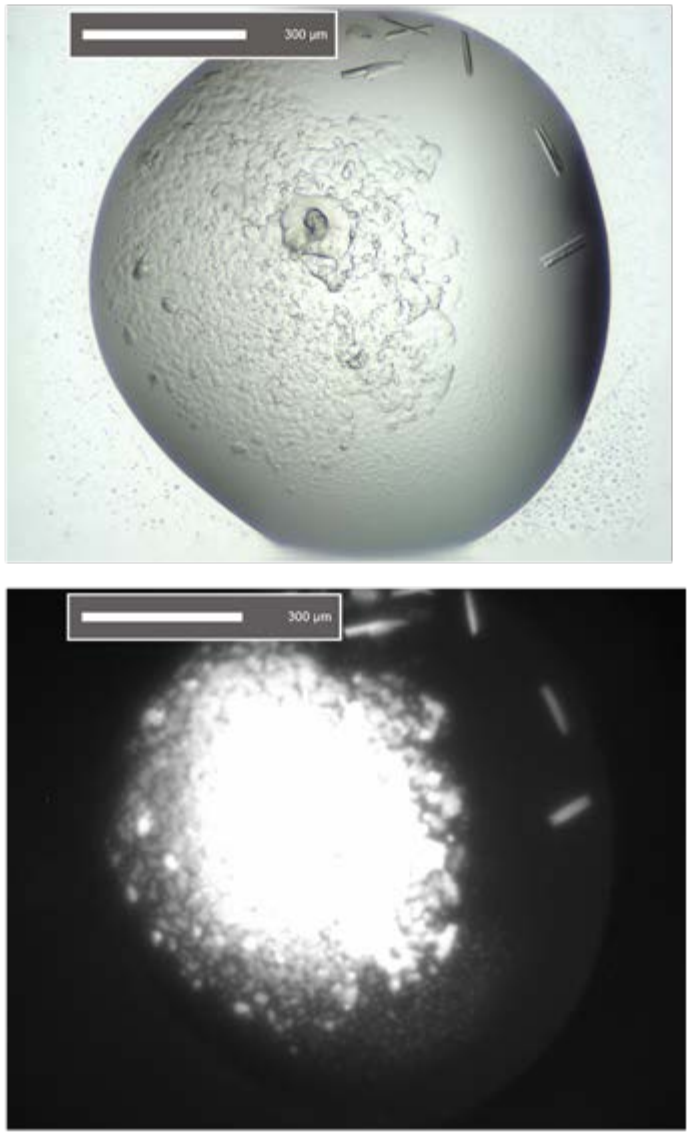


Figure S6. Crystals of VapBC-1. A) Visible light image and B) UV fluorescence image.

Table S1. Bacteria, plasmids and primers used in this study.

Strains	**Description**	**Source**
DH5α	F– Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (rK–, mK+) *pho*A *sup*E44 λ– *thi*-1 *gyr*A96 *rel*A1	Laboratory collection
LMG194	F^- Δ*lacX*74 *galE thi rpsL* Δ*phoA* (*PvuII*) Δ*ara*714 *leu*::Tn10	Invitrogen
SU101	*lexA*71::Tn*5*(Def) *sulA211* Δ(*lacIPOZYA*)*169*/F' $lacI^q$ *lacZ M15*::Tn*9*	M. Granger-Schnarr
SU202	*lexA*71::Tn*5*(Def) *sulA*211 Δ(*lacIPOZYA*)169/F' $lacI^q$ *lacZ* M15::Tn9	M. Granger-Schnarr
86-028NP Δ*vapBC-1*	Strain 86-028NP with the *vapBC-1* locus deleted	(1)
Plasmids	**Description**	**Source**
pBAD33	Highly regulated expression vector	(2)
pET24b	Regulated expression vector with His tag	Novagen
pBluescript SK(+)	Cloning vector	(1)
pSR658	WT LexA DBD fusion vector	(3)
pSR659	Mutant LexA DBD fusion vector	(3)
pSR660	WT LexA DBD fusion vector	(3)
pSR661	Mutant LexA DBD fusion vector	(3)
pDD686	*vapBC-1* in pET24b	(4)
pDD757	*vapBC-1* (*vapC-1* D6N*)* in pET24b	Unpublished
pDD758	*vapBC-1* (*vapC-1* D99N) in pET24b	(5)

pDD866	*vapB-1* in pSR658	(1)
pDD935	*vapB-1* in pTrcHisA	Unpublished
pDD946	*vapC-1* in pBAD33	Unpublished
pDD1058	5’ end of NTHI_RS09270 in pBluescript SK(+)	This work
pDD1063	3’ end of NTHI_RS09270 in pDD1058	This work
pDD1118	*vapBC-1* (*vapC-1* D6N/D99N) in pUC57	Genscript
pDD1119	*vapC-1* D6N/D99N in pBAD33	This work
pDD1120	*vapC-1* D99N in pSR658	This work
pDD1123	*vapC-1* D99N in pSR659	This work
pDD1126	*vapC-1* D6N in pSR661	This work
pDD1128	*vapC-1* D6N/D99N in pSR661	This work
pDD1132	*vapC-1* D6N in pBAD33	This work
pDD1133	*vapC-1* D99N in pBAD33	This work
pDD1140	NTHI_RS09270-targeted delivery vector	This work
pDD1150	*vapC-1* in pSR659	This work
pDD1152	*vapC-1* D6N in pET24b	This work
pDD1153	*vapC-1* D99N in pET24b	This work
pDD1154	*vapC-1* D6N/D99N in pET24b	This work
pDD1155	pDD1140 with the native *vapBC-1* promoter	This work
pDD1159	pDD1155 with *vapB-1vapC-1* D6N	This work
pDD1160	pDD1155 with *vapB-1vapC-1* D99N	This work
pDD1161	pDD1155 with *vapB-1vapC-1* D6N/D99N	This work
pDD1165	*vapBC-1* (*vapC-1* E43Q) in pCR2.1	Eurofins

pDD1166	*vapBC-1* (*vapC-1* E120Q) in pCR2.1	Eurofins
pDD1167	pDD1155 with *vapB-1vapC-1* E43Q	This work
pDD1168	pDD1155 with *vapB-1vapC-1* E120Q	This work
pDD1169	pDD1155 with *vapB-1vapC-1* wild-type	This work
pDD1170	*vapC-1* E43Q in pSR658	This work
pDD1173	*vapC-1* E43Q in pET24b	This work
pDD1174	*vapC-1* E120Q in pBAD33	This work
pDD1175	*vapC-1* E43Q in pBAD33	This work
pDD1177	*vapC-1* E120Q in pSR658	This work
pDD1178	*vapC-1* E43Q in pSR659	This work
pDD1179	*vapC-1* E120Q in pSR659	This work
pDD1194	*vapC-1* E43Q/D99N in pBAD33	This work
pDD1196	*vapC-1* E43Q/D99N in pBAD/*Myc*-HisA	This work
pDD1199	*vapC-1* E43Q/D99N in pSR658	This work
pDD1200	*vapC-1* E43Q/D99N in pSR659	This work
pDD1204	pDD1155 with *vapB-1vapC-1* E43Q/D99N	This work
Primers	**Description**	**Source**
D6NFor	[Phos]AACACCAATATCATTATTTATTTAATG	IDT
D6NRev	[Phos]TAACATATAAATCATAAATTTTCTCG	IDT
321LexFor	GAGGGAGCTCATGCTTACTAAAGTG	IDT
321LexRev	AACAGGTACCTCATAAATTTTCTCG	IDT
322LexFor	GAGAGAGCTCATGATTTATATGTTAG	IDT
322LexRev	TCAGGGTACCCTATTTTGTCCAATCTTGCC	IDT

pETD6NC1SacFor	AAAAGAGCTCTATGATTTATATGTTAAACACC	Eurofins
322Rev	TCAGAAGCTTCTATTTTGTCCAATCTTGCC	IDT
pBAD86C1SacFor	TTACGAGCTCAGGAGCGAGAAAATTTATG	Eurofins
XLacIFor	GTCATCTAGAAAACGCGCGAGGCAGC	Eurofins
XHisRev	TATTTGTCTAGAGGCAGTTCCCTACTCTCG	Eurofins
86C1NdeD6NFor	AAACATATGATTTATATGTTAAACAC	Eurofins
VapCXhoRev	GAATCTCGAGTTTTGTCCAATCTTGCC	Eurofins
86C1NdeFor	AAAACATATGATTTATATGTTAGAC	Eurofins
PermCSpeFor	AAATACTAGTAACACACACGCCATTCC	Eurofins
ErmCSpeRev	GTTAACTAGTGCAGTTATGCATCC	Eurofins
BC1PromBamFor	ACTAGGATCCATCATTTACTCATTGACTTGC	Eurofins
BC1PromNdeRev	TAAGCATATGTACCCTCTCGTATATAC	Eurofins
86B1NdeFor	GGTACATATGCTTACTAAAGTG	Eurofins
2009-1SacFor	TGCCGAGCTCGCAGGTAAGATGTCG	Eurofins
2009-1XbaRev	AAAATCTAGAGATGCACGTGCAACC	Eurofins
2009-2XhoFor	AAAACTCGAGGCTTGCATCTAATGC	Eurofins
2009-2KpnRev	AAAAGGTACCTCACTATCTTGTGCC	Eurofins

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