APPENDIX

Engineering a customizable antibacterial T6SS-based platform in *Vibrio natriegens*

Biswanath Jana[1], Kinga Keppel[1], and Dor Salomon[1,*]

[1] Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel.

***** For correspondence: dorsalomon@mail.tau.ac.il; Tel.: +972-3-6408583

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Appendix Table S1. Bacterial strains used in this study.

Strain name	Genotype	Comments	Source
Vibrio parahaemolyticus RIMD 2210633	Wild type	Used as a template for PCR amplifications	(Makino *et al*, 2003)
Vibrio parahaemolyticus RIMD 2210633 Δ*hcp1*	Δ*vp1393*	RIMD 2210633 derivative. Used as prey in bacterial competition assays	(Jana *et al*, 2019)
Vibrio parahaemolyticus POR1	Δ*tdhAS*	RIMD 2210633 derivative. Used as an attacker in competition assays, in secretion assays, and as a template for PCR amplifications	(Park *et al*, 2004)
Vibrio parahaemolyticus POR1 Δ*hcp1*	Δ*tdhAS*/Δ*vp1393*	RIMD 2210633 derivative. Used as an attacker in competition assays, in secretion assays, and as a template for PCR amplifications	(Salomon *et al*, 2013)
Vibrio parahaemolyticus POR1 Δ*vp1391*	Δ*tdhAS*/Δ*vp1391*	RIMD 2210633 derivative. Used as an attacker in competition assays, in secretion assays, and for quantitative RT- PCR	(Salomon *et al*, 2013)
Vibrio parahaemolyticus POR1 Δ*vp1407*	Δ*tdhAS*/Δ*vp1407*	RIMD 2210633 derivative. Used as an attacker in competition assays, in secretion assays, as a template for PCR amplifications, and for quantitative RT- PCR	(Salomon *et al*, 2013)
Vibrio parahaemolyticus POR1 Δ*tfoY*	Δ*tdhAS*/Δ*vp1028*	RIMD 2210633 derivative. Used as an attacker in competition assays, in secretion assays, and for quantitative RT- PCR	(Ben-Yaakov & Salomon, 2019)
Vibrio parahaemolyticus POR1 Δ3xRegulators	Δ*tdhAS*/Δ*vp1391*/Δ*vp1407*/Δ*vp1028*	RIMD 2210633 derivative. Used as an attacker in competition assays, in secretion assays, and for quantitative RT- PCR	This study
Vibrio parahaemolyticus POR1 *vp1415*^{AAA}	Δ*tdhAS*/*vp1415*^{AAA}	RIMD 2210633 derivative. Used as a template for PCR amplifications. Codons for histidines 563-4 in VP1415 were replaced by codons for alanines	This study

Vibrio parahaemolyticus 12-297/B Δ*hcp1*	Δ*b5c30_rs15290*	12-297/B derivative. Used as prey in competition assays	(Jana *et al*, 2019)
Vibrio parahaemolyticus 04.2548	Wild type	Used to prepare derivative strains	Obtained from Swapan Banerjee, (Banerjee *et al*, 2015)
Vibrio parahaemolyticus 04.2548 Δ*hcp1*	Δ*ba740_rs16850*	04.2548 derivative. Used as prey in competition assays	This study
Vibrio natriegens ATCC 14048	Wild type	Used as an attacker and prey in competition assays, as a template for PCR amplifications, and to prepare derivative strains	ATCC collection
Vibrio natriegens ATCC 14048 Δ*tfoY*	Δ*m272_rs24650*	ATCC 14048 derivative. Used as an attacker in competition assays	This study
Vnat$^{dns::araC+vp1409-7}$	The endogenous *dns* gene (*pn96_00865*) replaced by a cassette containing *araC* and P*bad*-controlled *V. parahaemolyticus vp1409-7*	ATCC 14048 derivative. Used to prepare derivative strains	This study
Vnat^{Reg}	The endogenous *dns* gene (*pn96_00865*) replaced by a cassette containing *araC* and P*bad*-controlled *V. parahaemolyticus vp1407*	ATCC 14048 derivative. Used as an attacker in bacterial competition	This study
Vibrio alginolyticus 12G01 Δ*hcp1*/Δ*hcp2*	Δ*v12g01_01540*/Δ*v12g01_07583*	12G01 derivative. Used as prey in competition assays	(Salomon *et al*, 2015)
Vibrio vulnificus CMCP6	Rifampicin-resistant parental strain	Used as prey in competition assays	Obtained from Karla Satchell
Aeromonas jandaei DSM 7311	Wild type (natural resistance to ampicillin)	Used to prepare derivative strains	DSMZ collection
Aeromonas jandaei DSM 7311 Δ*tssB*	Δ*bn1126_rs13720*	DSM 7311 derivative. Used as prey in competition assays	This study
Salmonella enterica serover choleraesuis strain SC-B67	Ampicillin, kanamycin, streptomycin and	Used as prey in competition assays	Obtained from Ohad Gal-Mor

	chloramphenicol-resistant strain		
Escherichia coli DH5α	K-12 derivative laboratory strain	Used as prey in competition assays	Lab stocks
Escherichia coli DH5α (λ pir)	K-12 derivative laboratory strain containing λ pir	Used for plasmid maintenance and cloning, and as prey in competition assays	Obtained from Eric V. Stabb

Appendix Table S2. Plasmids used in this study.

Plasmid name	Description	Purpose	Source
pYES1L	*E. coli*-yeast shuttle vector	Used to construct pCLTR	Novagen
pBAD33	A pBAD series expression plasmid encoding the Cm^R gene and the *p15A* origin of replication	Cm^R and *p15A ori* were amplified to construct pCLTR	Addgene
pUC18T-mini-Tn7T-Tp-dsRedExpress	TpR mini-Tn7 vector, mobilizable, for RFP tagging bacteria	*oriT* was amplified to construct pCLTR	Addgene
sfGFP-N1	Eukaryotic expression plasmid encoding superfolder GFP (sfGFP)	Used as a template to amplify *sfgfp*	Addgene
pEVS170	A mini-Tn5 delivery vector; Erm^R, Kan^R	Used to construct pTnp1222	(Lyell *et al*, 2008)
pTnp1222	Erm^R-containing plasmid; constructed by first integrating pEVS170 into the genome of *V. parahaemolyticus* POR1 at a random location, followed by digestion of the genomic DNA with HhaI and self-ligation of the products	Used to selectively grow *E. coli* prey on erythromycin-containing media when 5 different prey strains were mixed together during bacterial competition	This study
pBAD18	Bacterial expression vector with Gentamycin selection	Used to selectively grow *E. coli*, *V. natriegens*, *V. parahaemolyticus*, *V. alginolyticus*, and *A. jandaei* prey on media containing gentamicin during bacterial competition	Addgene
pBAD/*Myc*-His	Arabinose-inducible pBAD/*Myc*-His plasmid harboring a Kan^R cassette	Used for cloning and arabinose-inducible expression of various genes in *Vibrio*	(Salomon *et al*, 2013)
pVP1391	pBAD/*Myc*-His containing the CDS of *vp1391* in the MCS, in-frame with the C-terminal *Myc*-His tag	Used for arabinose-inducible expression of VP1391	This study

pVP1407	pBAD/*Myc*-His containing the CDS of *vp1407* in the MCS, out-of-frame with the C-terminal *Myc*-His tag	Used for arabinose-inducible expression of VP1407	This study
pTfoY	pBAD/*Myc*-His containing the CDS of *vp1028* in the MCS, in-frame with the C-terminal *Myc*-His tag	Used for arabinose-inducible expression of TfoY	(Ben-Yaakov & Salomon, 2019)
pVP1409-7	pBAD/*Myc*-His containing the CDS of *vp1409-7* in the MCS, out-of-frame with the C-terminal *Myc*-His tag	Used to place *vp1409-7* under a P*bad* promoter	This study
pBAD/*Myc*-His:PoNe/i$^{Vp\ 12\text{-}297/B}$	pBAD/*Myc*-His containing the *V. parahaemolyticus* 12-297/B effector and immunity module PoNe/i$^{Vp\ 12\text{-}297/B}$, consisting of *b5c30_rs14470-60*, in its MCS; the 3' gene is cloned in-frame with the C-terminal *Myc*-His tag of the plasmid	Used to place PoNe/i$^{Vp\ 12\text{-}297/B}$ under P*bad* control	(Fridman *et al*, 2020)
pBAD/*Myc*-His:Tme/i1$^{Vp\ BB22OP}$	pBAD/*Myc*-His containing the *V. parahaemolyticus* BB22OP effector and immunity module Tme/i1$^{Vp\ BB22OP}$, consisting of *vpbb_rs15030-35*, in its MCS; the 3' gene is cloned in-frame with the C-terminal *Myc*-His tag of the plasmid	Used to place Tme/i1$^{Vp\ BB22OP}$ under P*bad* control	(Fridman *et al*, 2020)
pBAD/*Myc*-His:VPA1263-Vti2$^{Vp\ RIMD}$	pBAD/*Myc*-His containing the *V. parahaemolyticus* RIMD 2210633 effector and immunity module VPA1263-Vti2$^{Vp\ RIMD}$ in its MCS; the 3' gene is cloned in-frame with the C-	Used to place VPA1263-Vti2$^{Vp\ RIMD}$ under P*bad* control	This study

	terminal *Myc*-His tag of the plasmid		
pBAD/*Myc*-His:Va2265-0$^{Va\ 12G01}$	pBAD/*Myc*-His containing the *V. alginolyticus* 12G01 effector and immunity module Va02265-0$^{Va\ 12G01}$, consisting of *v12g01_02265-0*, in its MCS; the 3' gene is cloned out-of-frame with the C-terminal *Myc*-His tag of the plasmid	Used to place Va02265-0$^{Va\ 12G01}$ under P*bad* control	(Salomon *et al*, 2015)
pBAD/*Myc*-His:TssB1-sfGFP	pBAD/*Myc*-His containing the *V. parahaemolyticus* RIMD 2210633 *tssB1* (*vp1402*) fused to a linker (AAAGGG) and *sfgfp*; 3' is cloned in-frame with the C-terminal *Myc*-His tag of the plasmid	Used to fuse TssB1 with sfGFP under P*bad* control	This study
pDM4	a Cm^R and $oriV_{R6K}$-containing suicide vector	To generate deletions, insertions, and replacements in *Vibrio* genomes	(O'Toole *et al*, 1996)
pDM4:*vp1391*	pDM4 containing 1 kb upstream and 1 kb downstream of *vp1391* in its MCS	Used to generate *V. parahaemolyticus* POR1 ∆3x Regulators	(Salomon *et al*, 2013)
pDM4:*vp1407*	pDM4 containing 1 kb upstream and 1 kb downstream of *vp1407* in its MCS	Used to generate *V. parahaemolyticus* POR1 ∆3x Regulators	(Salomon *et al*, 2013)
pDM4:*tfoY*	pDM4 containing 1 kb upstream and 1 kb downstream of the *V. parahaemolyticus vp1028* in its MCS	Used to generate *V. parahaemolyticus* POR1 ∆3x Regulators	(Ben-Yaakov & Salomon, 2019)
pDM4:*dns*^{Vnat}	pDM4 containing 1 kb upstream and 1 kb downstream of *V. natriegens dns* in its MCS	Used as platform to introduce cassettes that will replace *dns* in the *V. natriegens* genome	This study
pDM4:*dns*^{Vnat}*_vp1409-7*	pDM4:*dns*^{Vnat} into which the region spanning *araC* to the *rrnT1* terminator from	Used to replace the *V. natriegens dns* gene with a cassette including a constitutively expressed	This study

	pVP1409-7 was inserted between the upstream and the downstream regions of *dns*	*araC* and *vp1409-7* under P*bad* control	
pDM4:*dns*^{Vnat}*_vp1407*	pDM4:*dns*^{Vnat} into which the region spanning *araC* to the *rrnT1* terminator from pVP1407 was inserted between the upstream and downstream regions of *dns*	Used to replace the *V. natriegens dns* gene with a cassette including a constitutively expressed *araC* and *vp1407* under P*bad* control	This study
pDM4:*tfoY*^{Vnat}	pDM4 containing 1 kb upstream and 1 kb downstream of the *V. natrigens m272_rs24650* in its MCS	Used to delete *tfoY* in *V. natriegens*	This study
pDM4:*vp1415*	pDM4 containing a ~2.2 kb region encompassing 1.1 kb upstream and 1.1 kb downstream of the codons encoding histidines 563-4 in *V. parahaemolyticus vp1415*	Used as a template to replace the codons encoding histidines 563-4 in *V. parahaemolyticus vp1415* with alanines	This study
pDM4:*vp1415*^{AAA}	pDM4 containing a ~2.2 kb region encompassing 1.1 kb upstream and 1.1 kb downstream of the codons encoding histidines 563-4 in *V. parahaemolyticus vp1415* in which these two codons were mutated to encode alanines	Used to replace VP1415 histidines 563-4 with alanine in the *V. parahaemolyticus* genome	This study
pDM4:*tssB*$^{Aj\ DSM7311}$	pDM4 containing 1 kb upstream and 1 kb downstream of *A. jandaei bn1126_rs13720*	Used to delete *tssB* in *A. jandaei*	This study
pDM4:*hcp1*$^{Vp\ 04.2548}$	pDM4 containing 1 kb upstream and 1 kb downstream of *V. parahaemolyticus*	Used to delete *hcp1* in *V. parahaemolyticus* 04.2548	This study

	04.2548 *ba740_rs16850*		
pVSV209	Mobilizable plasmid contains $OriV_{pES213}$, $OriV_{R6K}$, Kan^R, constitutive *rfp*, and a promoterless Cm^R-*gfp* reporter	Used to carry TssB1-sfGFP and effector and immunity modules in *V. natriegens*	(Dunn *et al*, 2006)
pBJ209-araC	pVSV209 derivative in which the *rfp-gfp* cassette was replaced by the region spanning *araC* CDS to the *rnnT1* terminator from pBAD/*Myc*-His	Used as pEmpty for pVSV209-derived plasmids (pEI-x)	This study
pPoNe/$i^{Vp\ 12\text{-}297/B}$	pVSV209 derivative in which the *rfp-gfp* cassette was replaced by the region spanning the *Pbad* promoter to the *rnnT1* terminator from pBAD/*Myc*-His:PoNe/$i^{Vp\ 12\text{-}297/B}$	Used to express PoNe/$i^{Vp\ 12\text{-}297/B}$ in *V. natriegens*	This study
pTme/$i1^{Vp\ BB22OP}$	pVSV209 derivative in which the *rfp-gfp* cassette was replaced by the region spanning the *Pbad* promoter to the *rnnT1* terminator from pBAD/*Myc*-His: Tme/$i1^{Vp\ BB22OP}$	Used to express Tme/$i1^{Vp\ BB22OP}$ in *V. natriegens*	This study
pVPA1263-$Vti2^{Vp\ RIMD}$	pVSV209 derivative in which the *rfp-gfp* cassette was replaced by the region spanning the *Pbad* promoter to the *rnnT1* terminator from pBAD/*Myc*-His: VPA1263-$Vti2^{Vp\ RIMD}$	Used to express VPA1263-$Vti2^{Vp\ RIMD}$ in *V. natriegens*	This study
pVa2265-$0^{Va\ 12G01}$	pVSV209 derivative in which the *rfp-gfp* cassette was replaced by the region spanning the *Pbad* promoter to the *rnnT1* terminator from	Used to express Va2265-$0^{Va\ 12G01}$ in *V. natriegens*	This study

	pBAD/*Myc*-His: Va2265-0$^{Va\ 12G01}$		
pVPA1263-Vti2+Va02265-0	pVSV209 derivative in which the *rfp-gfp* cassette was replaced by the region spanning the *Pbad* promoter to the *rnnT1* terminator from pBAD/*Myc*-His: VPA1263-Vti2$^{Vp\ RIMD}$ followed by the same region from pBAD/*Myc*-His: Va2265-0$^{Va\ 12G01}$	Used to express VPA1263-Vti2$^{Vp\ RIMD}$ and Va2265-0$^{Va\ 12G01}$ in *V. natriegens* together	This study
pTssB1-sfGFP	pVSV209 derivative in which the *rfp-gfp* cassette was replaced by the region spanning the *Pbad* promoter to the *rnnT1* terminator from pBAD/*Myc*-His:TssB1-sfGFP	Used to express TssB1-sfGFP in *V. natriegens* together	This study
pCLTR	*E. coli*-yeast-*Vibrio* shuttle vector; mobilizable; it contains *Spec*^{R} and *Cm*^{R}	Used to capture VpT6SS1 and its derivatives	This study
pT6SS1	*vp1386-vp1420* (VpT6SS1) from *V. parahaemolyticus* in pCLTR	Used to transfer VpT6SS1 into *V. natriegens*	This study
pT6SS1^{Ind}	pT6SS1 lacking *vp1407*	Used as an inducible derivative of pT6SS1	This study
pT6SS1$^{Ind/\Delta hcp1}$	pT6SS1^{Ind} lacking *hcp1*	Used as a T6SS-inactive version of pT6SS1^{Ind}	This study
pT6SS1^{Effectorless}	pT6SS1^{Ind} lacking *vp1388-90* and carrying a mutated form of *vp1415* (*vp1415*^{AAA})	Used as an inducible derivative of pT6SS1 lacking endogenous active effector and immunity modules	This study
pT6SS1$^{Effectorless/\Delta hcp1}$	pT6SS1^{Effectorless} lacking *hcp1*	Used as a T6SS-inactive version of pT6SS1^{Effectorless}	This study

Appendix Table S3. Primers used in this study.

Primer name	Sequence (5' to 3')	Description
pCLTR_F1	GCGCAGCGGCGGCCGCGCTGATACCGCCGCTTGCTTTCGAATTTCTGCCATTC	Amplify *p15A ori* and Cm^R from pBAD33 to construct pCLTR
pCLTR_R1	GCCGCAGCCGAACGACCGAGATAAGCTGTCAAACATGAGCAG	Amplify *p15A ori* and Cm^R from pBAD33 to construct pCLTR
pCLTR_F2	TGCTCATGTTTGACAGCTTATCTCGGTCGTTCGGCTGCGGCG	Amplify *oriT* from pUC18-mini-Tn7T-Tp-dsRedExpress to construct pCLTR
pCLTR_R2	TCGCTCACTGACTTTAATTAACTGCGGCGAGGTATAGCGATTTTTTCGGTATATCC	Amplify *oriT* from pUC18-mini-Tn7T-Tp-dsRedExpress to construct pCLTR
T6SS_F	CCCTGTTTGCATTATGAATTAGTTACGCTAGGGGCGGCCGCAGTGAGCAGATAATGACTAAAAGATAC	Amplify a fragment of VpT6SS1 to construct pT6SS1
F1_R	GCCTTTACGGTAGCGCTTGTTGATTTC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F2_F	TGTAACAGCGTATGTTACAGATAAAAAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F2_R	TTTATTTATCTGGCTTCGTAACATTGC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F3_F	GAAGTATATTTGGCTTATAACAGTAAAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F3_R	ATACGACATTTCTGCGGCAGGTTG	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F4_F	CTTGGAAGCAGTATAACGAATCTCAAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F4_R	GTTGTTAGCGCGCGATAGC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F5_F	CAGTGTTAACTAATTACATCGTG	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F5_R	GACATAATTAGTTCCTTATCTAAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives

F6_F	GAAAAGTGGAAAGCAATTTATGAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F6_R	CTACTTCCGCACTCAATTCTCACC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F7_F	AGTTGCTCGAACGACATCCATGTTG	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F7_R	CCACTCTTCGCCAGTCTTATCCAG	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
F8_F	GCATTGCCTTACCACGCAGGTTAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 and its derivatives
T6SS_R	GCGCTCGGTCGTTCGGGTTCTATATTACCCTGGCGGCCGCAAGTGGTACTTCACTTGTTCAGCCG	Amplify a fragment of VpT6SS1 to construct pT6SS1
T6SS_2_R	AAGTGGTACTTCACTTGTTCAGCC	Amplify a fragment of VpT6SS1 to construct pT6SS1 derivatives
T6SS_2_F	AGTGAGCAGATAATGACTAAAAGATAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 derivatives
d88-90_R	GTTGAGATTCGTTATACTGCTTCCAAGCCAACATCGTAAACATGGTTGCGG	Amplify a fragment of VpT6SS1 to construct pT6SS1 derivatives
d88-90_F	CCGCAACCATGTTTACGATGTTGGCTTGGAAGCAGTATAACGAATCTCAAC	Amplify a fragment of VpT6SS1 to construct pT6SS1 derivatives
pT6SS1_Last_R	ATATCTATCATTATTTAGTTAGTAAAAACAAAGGAATTGTC	Amplify the pT6SS1 backbone to construct pT6SS1 derivatives
pT6SS1_Last_F	AAAGTGCCGAAAGGCGCTTTTCTTTTAATTACAATCC	Amplify the pT6SS1 backbone to construct pT6SS1 derivatives
pBADfix_GIB_F	AAGCTTGGGCCCGAACAAAAAC	Amplify the pBAD/*Myc*-His backbone for cloning using the Gibson assembly method
pBADfix_GIB_R	CATGGTTAATTCCTCCTGTTAG	Amplify the pBAD/*Myc*-His backbone for cloning using the Gibson assembly method
pBAD_vp1391_F	GCTAACAGGAGGAATTAACCATGCGTTCAGCTAACCATAGCG	Amplify *vp1391* to construct pVP1391
pBAD_vp1391_R	TTTTGTTCGGGCCCAAGCTTACCTGCCGCAGAAATGTCGTATTTC	Amplify *vp1391* to construct pVP1391

pBAD_vp1407_F	GCTAACAGGAGGAATTAACCATGATTTCTAATATGACTTTACAAG	Amplify *vp1407* to construct pVP1407
pBAD_vp1407_R_STOP	TTTTGTTCGGGCCCAAGCTTCTATCGACTGATCGGCTGATGAAG	Amplify *vp1407* to construct pVP1407 and pVP1409-7
pBAD_VP1409_F	GCTAACAGGAGGAATTAACCATGGAACTTAACATTATGTTTTTCAG	Amplify *vp1409* to construct pVP1409-7
VN_Dns_Up_F	CGCGAGCTCTTGCCCTTCTTAGTGATTGGGTC	Amplify the 1 kb upstream of *V. natriegens dns* (PN96_00865) to construct pDM4:*dns*^{Vnat}
VN_Dns_Up_R	ATAAGAATGCGGCCGCAGTTAAAGTCTTTAAAAAGTATGAC	Amplify the 1 kb upstream of *V. natriegens dns* (PN96_00865) to construct pDM4:*dns*^{Vnat}
VN_Dns_Dn_F	ATAAGAATGCGGCCGCCCAGCCGATAAATGGGAATGC	Amplify the 1 kb downstream of *V. natriegens dns* (PN96_00865) to construct pDM4:*dns*^{Vnat}
VN_Dns_Dn_R	ACGCGTCGACGGCAAAGCTAGAGTCTTTCTTG	Amplify the 1 kb downstream of *V. natriegens dns* (PN96_00865) to construct pDM4:*dns*^{Vnat}
Not1_Fix_F	ATAAGAATGCGGCCGCATGCATAATGTGCCTGTCAAATGG	Amplify the region spanning *araC* and *rrnT1* from pBAD/*Myc*-His derivatives to construct pDM4:*dns*^{Vnat}*_vp1407* or pDM4:*dns*^{Vnat}*_vp1409-7*
Not1_Fix_R	ATAAGAATGCGGCCGCTAGAAACGCAAAAAGGCCATCCG	Amplify the region spanning *araC* and *rrnT1* from pBAD/*Myc*-His derivatives to construct pDM4:*dns*^{Vnat}*_vp1407* or pDM4:*dns*^{Vnat}*_vp1409-7*
VN_TfoY_Up_F	CGCGAGCTCTATCGAATTGGCAGAGTACTTC	Amplify 1 kb upstream of *V. natriegens tfoY* (*m272_rs24650*) to construct pDM4:*tfoY*^{Vnat}
VN_TfoY_Up_R	CGCGGATCCGATGTGTTATCTCGGTGTAATTATTATTACG	Amplify 1 kb upstream of *V. natriegens tfoY* (*m272_rs24650*) to construct pDM4:*tfoY*^{Vnat}
VN_TfoY_Dn_F	CGCGGATCCTCTGTAGGTATAAACCTATCGTAC	Amplify 1 kb downstream of *V. natriegens tfoY*

		(*m272_rs24650*) to construct pDM4:*tfoY*^{Vnat}
VN_TfoY_Dn_R	ACGCGTCGACAATGGTAGTTCCCCCTTCAATCCG	Amplify 1 kb downstream of *V. natriegens tfoY* (*m272_rs24650*) to construct pDM4:*tfoY*^{Vnat}
VPA1263_F	TAACAGGAGGAATTAACCATGTCCTATAAAGTTCTTCCACTG	Amplify the *vpa1263-vti2* effector and immunity module from *V. parahaemolyticus* RIMD 2210633 to construct pBAD/*Myc*-His:VPA1263-Vti2$^{Vp\ RIMD}$
Vit2_R	TTTTGTTCGGGCCCAAGCTTCTCTTTGAAGCAAGGTTCTC	Amplify the *vpa1263-vti2* effector and immunity module from *V. parahaemolyticus* RIMD 2210633 to construct pBAD/*Myc*-His:VPA1263-Vti2$^{Vp\ RIMD}$
pVSV_F	AAACTCTTTTGTTTATTTTCCCCGGGAATTCCCATGTCAGCCG	Amplify the pVSV209 backbone for cloning using the Gibson assembly method
pVSV_R	GGCGGGAGTATGAAAAGGAGTGAGCTAACTCACATTAATTG	Amplify the pVSV209 backbone for cloning using the Gibson assembly method
pBADfix_R_MCS	AAAATAAACAAAAGAGTTTGTAGAAAC	Amplify the region between the P*bad* promoter and the *rnnT1* terminator from pBAD/*Myc*-His derivatives to clone into pVSV209 using the Gibson assembly method
pBADfix_F_MCS	CTTTTCATACTCCCGCCATTCAGAG	Amplify the region between the P*bad* promoter and the *rnnT1* terminator from pBAD/*Myc*-His derivatives to clone into pVSV209 using the Gibson assembly method
pDM4_Gib_VP1415_F	GTGGAATCCCGGGAGAGCTCAGAGATATAGAGCCAATCCAAAAG	Amplify the ~2.2 kb region flanking the *vp1415* codons encoding histidines 563-4 from *V. parahaemolyticus* POR1 to construct pDM4:*vp1415*

pDM4_Gib_VP1415_R	TAGCGGAGTGTATATCAAGCTTTTAAATTTTCATTCCAAAACCAATG	Amplify the ~2.2 kb region flanking the *vp1415* codons encoding histidines 563-4 from *V. parahaemolyticus* POR1 to construct pDM4:*vp1415*
VP1415_HH_AA_F	GGAGCAGTGCAGGCTGCAGCTCTTATTCCTAAAAATGCATTC	Site-directed mutagenesis to replace histidines 563-4 of VP1415 with alanines
VP1415_HH_AA_R	CACTGCTCCCGCCCCGTGCCACCAAGGGTGC	Site-directed mutagenesis to replace histidines 563-4 of VP1415 with alanines
VP1386_F_RTPCR	AAGGCGTGGTGAACTTCAGT	Determine the expression of *vp1386* in quantitative RT-PCR
VP1386_R_RTPCR	CAAAGCTACTCTGCCCACCT	Determine the expression of *vp1386* in quantitative RT-PCR
VP1388_F_RTPCR	ACTGGGTTGCGTTGTCTTCT	Determine the expression of *vp1388* in quantitative RT-PCR
VP1388_R_RTPCR	CTGGACTTGGCTCAGAAGGG	Determine the expression of *vp1388* in quantitative RT-PCR
VP1392_F_RTPCR	ACCCTATTGCCGTTGGTGAG	Determine the expression of *vp1392* in quantitative RT-PCR
VP1392_R_RTPCR	ATGATCGGTGTTGGCGAGTT	Determine the expression of *vp1392* in quantitative RT-PCR
VP1393_F_RTPCR	AGCTGATTGATGCGCTATTGG	Determine the expression of *vp1393* in quantitative RT-PCR
VP1393_R_RTPCR	TCGTCGTTACCAGCTGTACC	Determine the expression of *vp1393* in quantitative RT-PCR
VP1406_F_RTPCR	CGGGCAGCAGACGATTCTAT	Determine the expression of *vp1406* in quantitative RT-PCR
VP1406_R_RTPCR	ACTCAATTCGCTCCTGGTGG	Determine the expression of *vp1406* in quantitative RT-PCR
VP1409_F_RTPCR	CCAGTCAAAGTGAGCCAGGT	Determine the expression of *vp1409* in quantitative RT-PCR
VP1409_R_RTPCR	GCAATGTTGGCAAAGACCGT	Determine the expression of *vp1409* in quantitative RT-PCR

VP1414_F_RT-pcr	TTGGAGCCAGAACAGTTTGC	Determine the expression of *vp1414* in quantitative RT-PCR
VP1414_R_RT-pcr	TGCTAGCTTTTCGTTACCGC	Determine the expression of *vp1414* in quantitative RT-PCR
16s rRNA_F RT-PCR	GACACGGTCCAGACTCCTAC	Determine the expression of 16s rRNA in quantitative RT-PCR
16s rRNA_R RT-PCR	GGTGCTTCTTCTGTCGCTAAC	Determine the expression of 16s rRNA in quantitative RT-PCR
Aero_tssB_SalI_UP_F	CAAAGTCGACATGGTCTCGCCCTGAAAC	Amplify the 1 kb upstream of *A. jandaei* DSM 7311 *tssB* (*bn1126_rs13720*) to construct pDM4:*tssB*^{Aj} DSM7311
Aero_tssB_KpnI_UP_R_	CAATGGTACCGCTCTAAATACCAAGTTAAGGG	Amplify the 1 kb upstream of *A. jandaei* DSM 7311 *tssB* (*bn1126_rs13720*) to construct pDM4:*tssB*^{Aj} DSM7311
Aero_tssB_KpnI_DN_F	CAATGGTACCTCGCCGGGTTGGATTGACTGAAC	Amplify the 1 kb downstream of *A. jandaei* DSM 7311 *tssB* (*bn1126_rs13720*) to construct pDM4:*tssB*^{Aj} DSM7311
Aero_tssB_XbaI_DN_R	CAAATCTAGAAGATCGTGGATGGCACCAC	Amplify the 1 kb downstream of *A. jandaei* DSM 7311 *tssB* (*bn1126_rs13720*) to construct pDM4:*tssB*^{Aj} DSM7311
TssB-link-GFP_F1	GCTAACAGGAGGAATTAACCATGTCACGTGACGGCTCGGTG	Amplify *tssB1* (*vp1402*) from *V. parahaemolyticus* POR1 to construct pBAD/*Myc*-His:TssB1-sfGFP
TssB-link-sfGFP_R1	ACCACCACCAGCAGCAGCCTCTTCTTTCGCGTCTTGGTC	Amplify *tssB1* (*vp1402*) from *V. parahaemolyticus* POR1 to construct pBAD/*Myc*-His:TssB1-sfGFP
TssB-link-sfGFP_F2	GCTGCTGCTGGTGGTGGTATGGTGAGCAAGGGCGAGGAG	Amplify *sfgfp* from sfGFP-N1 plasmid to construct pBAD/*Myc*-His:TssB1-sfGFP
TssB-link-sfGFP_R2	TTTTGTTCGGGCCCAAGCTTCTTGTACAGCTCGTCCATGCC	Amplify *sfgfp* from sfGFP-N1 plasmid to construct

		pBAD/*Myc*-His:TssB1-sfGFP
Hcp1_UP_F_SacI	CAAAGAGCTCCTGTCGTGAACTTGCTCAG	Amplify 1 kb upstream of *V. parahaemolyticus* 04.2548 *hcp1* (*ba740_rs16850*) to construct pDM4:*hcp1*^{Vp} 04.2548
Hcp1_UP_R_BamHI	CAACGGATCCCGCTATTTCCTTTTCTAAAATCTG	Amplify 1 kb upstream of *V. parahaemolyticus* 04.2548 *hcp1* (*ba740_rs16850*) to construct pDM4:*hcp1*^{Vp} 04.2548
Hcp1_DN_F_BamHI	CACCGGATCCTTGCTTTTTGCGTAAAGATTCAGG	Amplify 1 kb downstream of *V. parahaemolyticus* 04.2548 *hcp1* (*ba740_rs16850*) to construct pDM4:*hcp1*^{Vp} 04.2548
Hcp1_DN_R_SalI	CAAAGTCGACCTGTAACCAGACGCCAAACG	Amplify 1 kb downstream of *V. parahaemolyticus* 04.2548 *hcp1* (*ba740_rs16850*) to construct pDM4:*hcp1*^{Vp} 04.2548

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