

S5 Table. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype	Source
<i>E. coli</i> strains		
DH5-α λpir	F' endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1 Δ(argF-lacZYA) U169(Φ80lacDM15) λ pir	BCCM Gene Corner
CC118 λ pir	Δ(ara-leu) araD ΔlacX74 galE galK phoA20 thi-1 rpsE rpoB argE(Am) recA1 λ pir	(Herrero et al., 1990)
S17-1 λ pir	Tpr Smr recA, thi, pro, rK- mK+ RP4:2-Tc:MuKm Tn7 λ pir	(de Lorenzo and Timmis, 1994)
<i>V. cholerae</i> strains		
FY_Vc_2	Rugose variant, <i>V. cholerae</i> O1 El Tor A1552, Rif ^r	(Yildiz and Schoolnik, 1999)
FY_Vc_4327	Δvps-IΔvps-II	(Fong et al., 2010)
FY_Vc_3679	ΔvpsU	(Fong et al., 2010)
FY_Vc_3384	ΔvpsO	(Fong et al., 2010)
FY_Vc_9329	ΔvpsUΔvpsO	This study
FY_Vc_11467	FY_Vc3679_ΔvpsU Tn7::native promoter-vpsU, Gm ^r	This study
FY_Vc_13255	FY_Vc3384_ΔvpsO Tn7::lacI promoter from pMalc-5X-vpsO, Gm ^r	This study
FY_Vc_11564	vpsU-C12S	This study
FY_Vc_11907	vpsO-K551A	This study
FY_Vc_13068	FY_Vc_3679 vpsO-K551A	This study
FY_Vc_11629	FY_Vc_11512_RΔctxABΔvpsO pBAD B vpsO-myc/his, Rif ^r , Amp ^r	This study
FY_Vc_11619	vpsO-Y727F	This study
FY_Vc_12022	vpsO-Y717F, Y723F	This study
FY_Vc_13398	vpsO-Y717F, Y720F, Y721F	This study
FY_Vc_13369	vpsO-Y723F, Y726F, Y727F	This study
FY_Vc_11892	vpsO-Y720F, Y721F, Y726F, Y727F	This study
FY_Vc_12031	vpsO-Y72F	This study
FY_Vc_12815	vpsO-Y72F, Y150F, Y285F	This study
FY_Vc_12230	vpsO-E148A, R151A, R154A	This study
FY_Vc_12236	vpsO-E519A, R522A, R525A	This study
FY_Vc_11589	vpsO-Y717F	This study
FY_Vc_11592	vpsO-Y720F	This study
FY_Vc_11610	vpsO-Y721F	This study
FY_Vc_11613	vpsO-Y723F	This study
FY_Vc_11616	vpsO-Y726F	This study
FY_Vc_13366	vpsO-Y720F, Y721F	This study
FY_Vc_13395	vpsO-Y726F, Y727F	This study

FY_Vc_12034	<i>vpsO-Y150F</i>	This study
FY_Vc_12037	<i>vpsO-Y285F</i>	This study
FY_Vc_12440	<i>vpsO-Y150F, Y285F</i>	This study
FY_Vc_12609	<i>vpsO-Y72F, Y150F</i>	This study
FY_Vc_12995	<i>vpsO-Y72F, Y285F</i>	This study
FY_Vc_1	<i>V. cholerae</i> O1 El Tor A1552, Rif ^r	(Yildiz and Schoolnik, 1999)
FY_Vc_15677	$\Delta vpsO$	This study
FY_Vc_15692	<i>vpsO-K551A</i>	This study
FY_Vc_6271	$\Delta vpsU$ pBAD B <i>vpsU-myc/his</i>	This study
FY_Vc_10767	$\Delta vpsA$ pBAD B- <i>vpsA-myc/his</i>	This study
FY_Vc_10771	$\Delta vpsB$ pBAD B- <i>vpsB-myc/his</i>	This study
FY_Vc_6274	$\Delta vpsC$ pBAD B- <i>vpsC-myc/his</i>	This study
FY_Vc_10777	$\Delta vpsD$ pBAD B- <i>vpsD-myc/his</i>	This study
FY_Vc_10781	$\Delta vpsE$ pBAD B- <i>vpsE-myc/his</i>	This study
FY_Vc_10785	$\Delta vpsF$ pBAD B- <i>vpsF-myc/his</i>	This study
FY_Vc_6282	$\Delta vpsG$ pBAD B- <i>vpsG-myc/his</i>	This study
FY_Vc_10791	$\Delta vpsH$ pBAD B- <i>vpsH-myc/his</i>	This study
FY_Vc_10795	$\Delta vpsI$ pBAD B- <i>vpsI-myc/his</i>	This study
FY_Vc_10799	$\Delta vpsJ$ pBAD B- <i>vpsJ-myc/his</i>	This study
FY_Vc_10803	$\Delta vpsK$ pBAD B- <i>vpsK-myc/his</i>	This study
FY_Vc_10807	$\Delta vpsL$ pBAD B- <i>vpsL-myc/his</i>	This study
FY_Vc_10811	$\Delta vpsM$ pBAD B <i>vpsM-myc/his</i>	This study
FY_Vc_10826	$\Delta vpsN$ pBAD B- <i>vpsN-myc/his</i>	This study
FY_Vc_10830	$\Delta vpsO$ pBAD B- <i>vpsO-myc/his</i>	This study
FY_Vc_10834	$\Delta vpsP$ pBAD B- <i>vpsP-myc/his</i>	This study
FY_Vc_14088	$\Delta vpsQ$ pBAD B- <i>vpsQ-myc/his</i>	This study
FY_Vc_240	FY_Vc_2_Tn7::gfp	(Beyhan and Yildiz, 2007)
FY_Vc_6230	FY_Vc_3679_Tn7::gfp	This study
KDV1276	Rugose pNUT542	This study
KDV1277	$\Delta vpsO$ pNUT542	This study
KDV1278	$\Delta vpsU$ pNUT542	This study
KDV1279	<i>vpsO-K551A</i> pNUT542	This study
KDV1280	<i>vpsU-C12S</i> pNUT542	This study
KDV1281	<i>vpsO-Y727F</i> pNUT542	This study
KDV1282	<i>vpsO-Y720F, Y721F, Y726F, Y727F</i> pNUT542	This study
KDV1283	<i>vpsO-Y717F, Y720F, Y721F</i> pNUT542	This study
KDV1284	<i>vpsO-Y723F, Y726F, Y727F</i> pNUT542	This study
KDV1285	<i>vpsO-Y72F</i> pNUT542	This study
KDV1286	<i>vpsO-Y72F, Y150F, Y285F</i> pNUT542	This study
KDV1287	<i>vpsO-E148A, R151A, R154A</i> pNUT542	This study
KDV1288	<i>vpsO-E519A, R522A, R525A</i> pNUT542	This study
FY_8902	FY_Vc_2_pMMB67EH	(Giglio et al., 2013)

FY_15745	FY_Vc_3384_pMMB67EH	This study
FY_15747	FY_Vc_3384_pMMB67EH-vpsO	This study
Plasmids		
p2GT	pET vector, IPTG-inducible vector for expression of recombinant proteins with N-terminal His6-GST tags, cleavable by TEV protease, Amp ^r	QB3 MacroLab
pFY_4443	p2GT-vpsU	This study
pFY_4556	p2GT-vpsU-C12S	This study
pFY_5813	p2GT-vpsO ⁴⁷³	This study
pGEV	pGEX-4T vector with an engineered TEV protease site, IPTG-inducible vector for expression of recombinant proteins with N-terminal GST tag, cleavable by TEV protease, Amp ^r	GE Healthcare
pFY_5484	pGEV-vpsO ⁵⁰³	This study
pFY_5495	pGEV- vpsO ⁵⁰³ -K551A	This study
pFY_5473	pGEV- vpsO ⁵⁰³ -Y717A, Y720A, Y721A, Y723A, Y726A, Y727A	This study
pFY_4758	pGEV- vpsO ⁵⁰³ -Y720A, Y721A, Y726A, Y727A	This study
pGP704sacB28	pGP704 derivative, mob/oriT sacB, Amp ^r	G. Schoolnik
pFY_4773	pGP-vpsU-C12S, for chromosomal point mutation	This study
pFY_4505	pGP-vpsO-K551A, for chromosomal point mutation	This study
pFY_5469	pGP-vpsO-Y727F, for chromosomal point mutation	This study
pFY_4520	pGP-vpsO-Y717F, Y723F, for chromosomal point mutation	This study
pFY_5516	pGP-vpsO-Y717F, Y720F, Y721F, for chromosomal point mutation	This study
pFY_5509	pGP-vpsO-Y723F, Y726F, Y727F, for chromosomal point mutation	This study
pFY_4500	pGP-vpsO-Y720F, Y721F, Y726F, Y727F, for chromosomal point mutation	This study
pFY_4516	pGP-vpsO-Y72F, for chromosomal point mutation	This study
pFY_5068	pGP-vpsO-Y72F, Y150F, Y285F, for chromosomal point mutation	This study
pFY_4595	pGP-vpsO-E148A, R151A, R154A, for chromosomal point mutation	This study
pFY_4597	pGP-vpsO-E519A, R522A, R525A, for	This study

	chromosomal point mutation	
pFY_1434	pMAL-c5X, IPTG-inducible vector designed to produce maltose-binding protein fusions, Ampr	New England Biolabs
pFY_5472	pGP-vpsO-Y717F, for chromosomal point mutation, Ampr	This study
pFY_5467	pGP-vpsO-Y720F, for chromosomal point mutation, Ampr	This study
pFY_5468	pGP-vpsO-Y721F, for chromosomal point mutation, Ampr	This study
pFY_5471	pGP-vpsO-Y723F, for chromosomal point mutation, Ampr	This study
pFY_5470	pGP-vpsO-Y726F, for chromosomal point mutation, Ampr	This study
pFY_5508	pGP-vpsO-Y720F, Y721F , for chromosomal point mutation, Ampr	This study
pFY_5515	pGP-vpsO-Y726F, Y727F , for chromosomal point mutation, Ampr	This study
pFY_4510	pGP-vpsO-Y150F, for chromosomal point mutation, Ampr	This study
pFY_4512	pGP-vpsO-Y285F, for chromosomal point mutation, Ampr	This study
pFY_4615	pGP-vpsO-Y150F, Y285F, for chromosomal point mutation, Ampr	This study
pFY_4613	pGP-vpsO-Y72F, Y150F , for chromosomal point mutation, Ampr	This study
pFY_4617	pGP-vpsO-Y72F, Y285F, for chromosomal point mutation, Ampr	This study
pNUT542	P _{tac} _sfgfp expression plasmid, Gm ^r	(Fong et al., 2017)

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