

SUPPLEMENTARY INFORMATION

Supplementary Table 1 | SaPlbov1 complementation analysis.

Strain	Phage	Plasmid expressing	No CdCl ₂		5 μM CdCl ₂	
			SaPlbov1 titre ^a	Phage titre ^b	SaPlbov1 titre ^a	Phage titre ^b
JP1794	φ11	-	2.0 × 10 ⁷	6.4 × 10 ⁷	1.0 × 10 ⁷	6.6 × 10 ⁶
JP4125	φ11 Δdut	-	200	1.1 × 10 ⁹	120	1.4 × 10 ⁹
JP6789	φ11 Δdut	dut φ11	2.4 × 10 ⁶	5.1 × 10 ⁷	3.4 × 10 ⁷	1.4 × 10 ⁷
JP6790	φ11 Δdut	dut φ11 D81A	1.2 × 10 ⁷	8.2 × 10 ⁷	5.6 × 10 ⁷	9.5 × 10 ⁶
JP6797	φ11 Δdut	dut 80α	550	1.7 × 10 ⁸	2.3 × 10 ⁷	5.6 × 10 ⁸
JP6791	φ11 Δdut	dut PH15	230	4.0 × 10 ⁸	110	1.9 × 10 ⁹
JP6796	φ11 Δdut	dut φ11 ^{fs}	300	1.6 × 10 ⁹	70	2.3 × 10 ⁹
JP6794	φ11 Δdut	dut 80α K8R, K12E	110	9.1 × 10 ⁸	2.2 × 10 ⁷	4.9 × 10 ⁸
JP6800	φ11 Δdut	dut 80α K12E	70	2.9 × 10 ⁹	2.1 × 10 ⁷	8.9 × 10 ⁸
JP6793	φ11 Δdut	dut 80α K8R	140	9.0 × 10 ⁸	2.4 × 10 ⁷	8.2 × 10 ⁸
JP6795	φ11 Δdut	dut φ11 R8K, E12K	1.3 × 10 ⁶	1.0 × 10 ⁷	4.5 × 10 ⁷	6.5 × 10 ⁶
JP6798	φ11 Δdut	dut φ11 R8K	2.0 × 10 ⁶	3.3 × 10 ⁷	3.3 × 10 ⁷	6.7 × 10 ⁶
JP6799	φ11 Δdut	dut φ11 E12K	1.9 × 10 ⁶	8.1 × 10 ⁷	7.4 × 10 ⁷	1.3 × 10 ⁶
JP3603	80α	-	4.0 × 10 ⁷	3.1 × 10 ⁸	3.5 × 10 ⁷	1.9 × 10 ⁸
JP6132	80α Δdut	-	140	5.2 × 10 ¹⁰	120	8.2 × 10 ⁹
JP6881	80α Δdut	dut 80α	520	4.8 × 10 ¹⁰	1.8 × 10 ⁷	1.3 × 10 ⁸
JP6879	80α Δdut	dut 80α D95E	220	2.1 × 10 ¹⁰	180	1.3 × 10 ¹⁰

^aNo. transductants x ml of induced culture, using RN4220 as recipient strain.^bNo. plaques x ml of induced culture, using RN4220 as recipient strain.

Supplementary Table 2 | SaPlbov2 complementation analysis.

Strain	Prophage	Plasmid	Induction ^a	SaPlbov2 titre ^b
JP6764	80 α	-		1.7×10^7
JP6215	80 α ΔORF15	-		5.4×10^2
JP6765	80 α ΔORF15	ORF15 80 α	+	5.5×10^4
JP6765	80 α ΔORF15	ORF15 80 α	+++ ^a	2.7×10^6
JP6766	80 α ΔORF15	ORF73 phi85	+	2.2×10^2
JP6766	80 α ΔORF15	ORF73 phi85	+++ ^a	8.3×10^5
JP2131	φ11	-		50
JP6767	φ11	ORF15 80 α	+	1.4×10^6
JP6767	φ11	ORF15 80 α	+++ ^a	6.5×10^6
JP6768	φ11	ORF73 phi85	+	6.1×10^2
JP6768	φ11	ORF73 phi85	+++ ^a	7.0×10^5

^a+: basal induction (without CdCl₂); +++: using 5 μM CdCl₂ as inducer^bNo. transductants x ml of induced culture, using RN4220 as recipient strain.**Supplementary Table 3 | dUTPase activity.**

Phage	Protein ^a	Activity ^b
φ11	wt	0.057
φ11	D81A	0
80 α	wt	0.033
80 α	D95E	0.024
PH15	wt	0.044

^aHis(6)-Dut protein purified from JP4560 (φ11 WT), JP4559 (φ11 D81A), JP5359 (80 α WT), JP5358 (80 α D95E), JP5527 (PH15)^bMeasured as ΔOD₃₆₀/min, using 0.1 μg of purified protein. Variation was within ±5% in all cases.

Supplementary Table 4 | Strains and plasmids used in this study.

Strains	Description	Reference
RN4220	Restriction-defective derivate of RN450	16
RN450	NCTC8325 cured of φ11, φ12 and φ13	17
RN981	RN450 <i>recA</i> -mutant	18
BL21(DE3)	<i>E. coli</i> expression strain	Stratagene
RN451	RN450 φ11	17
RN10359	RN450 80α	19
JP4025	RN451 Δ <i>dut</i>	This work
JP6032	RN10359 Δ <i>dut</i>	This work
JP6022	RN10359 Δ <i>sri</i>	This work
JP6015	RN10359 ΔORF15	This work
JP1794	RN451 SaPIbov1 <i>tst</i> :: <i>tetM</i>	This work
JP2131	RN451 SaPIbov2 <i>bap</i> :: <i>tetM</i>	20
JP4125	RN451 Δ <i>dut</i> SaPIbov1 <i>tst</i> :: <i>tetM</i>	This work
JP3603	RN10359 SaPIbov1 <i>tst</i> :: <i>tetM</i>	This work
JP6132	RN10359 Δ <i>dut</i> SaPIbov1 <i>tst</i> :: <i>tetM</i>	This work
JP6879	JP6132 pJP685	This work
JP6881	JP6132 pJP919	This work
JP6122	RN10359 Δ <i>sri</i> SaPIbov1 <i>tst</i> :: <i>tetM</i>	This work
JP6115	RN10359 ΔORF15 SaPIbov1 <i>tst</i> :: <i>tetM</i>	This work
JP6764	RN10359 SaPIbov2 <i>bap</i> :: <i>tetM</i>	This work
JP6232	RN10359 Δ <i>dut</i> SaPIbov2 <i>bap</i> :: <i>tetM</i>	This work
JP6222	RN10359 Δ <i>sri</i> SaPIbov2 <i>bap</i> :: <i>tetM</i>	This work
JP6215	RN10359 ΔORF15 SaPIbov2 <i>bap</i> :: <i>tetM</i>	This work
JP3602	RN10359 SaPI1 <i>tst</i> :: <i>tetM</i>	This work
JP6332	RN10359 Δ <i>dut</i> SaPI1 <i>tst</i> :: <i>tetM</i>	This work
JP6322	RN10359 Δ <i>sri</i> SaPI1 <i>tst</i> :: <i>tetM</i>	This work
JP6315	RN10359 ΔORF15 SaPI1 <i>tst</i> :: <i>tetM</i>	This work
JP6772	RN4220 SaPIbov1 <i>tst</i> :: <i>tetM</i> Δ <i>stl</i>	This work
JP6773	RN981 SaPIbov1 <i>tst</i> :: <i>tetM</i> pJP813	This work
JP5468	RN4220 pJP674 pJP653	This work
JP5469	RN4220 pJP674 pJP658	This work
JP6765	RN10359 ΔORF15 SaPIbov2 <i>bap</i> :: <i>tetM</i> pJP751	This work
JP6766	RN10359 ΔORF15 SaPIbov2 <i>bap</i> :: <i>tetM</i> pJP752	This work
JP6767	RN451 SaPIbov2 <i>bap</i> :: <i>tetM</i> pJP751	This work
JP6768	RN451 SaPIbov2 <i>bap</i> :: <i>tetM</i> pJP752	This work
JP6287	RN4220 SaPIbov2 <i>bap</i> :: <i>tetM</i> pJP751	This work
JP6294	RN4220 SaPIbov2 <i>bap</i> :: <i>tetM</i> pCN51 (empty)	This work
JP6760	BL21(DE3) pJP756	This work
JP6761	BL21(DE3) pJP757	This work

Strains	Description	Reference
JP6763	BL21(DE3) pJP758	This work
JP4560	BL21(DE3) pJP666	This work
JP4559	BL21(DE3) pJP667	This work
JP5357	BL21(DE3) pJP755	This work
JP5359	BL21(DE3) pJP753	This work
JP6762	BL21(DE3) pJP811	This work
JP5358	BL21(DE3) pJP754	This work
JP5527	BL21(DE3) pJP675	This work
JP6774	RN4220 Δ spha SaPlbov1 <i>tst::tetM</i>	This work
JP6789	JP6774 pJP813	This work
JP6790	JP6774 pJP814	This work
JP6791	JP6774 pJP815	This work
JP6793	JP6774 pJP817	This work
JP6794	JP6774 pJP818	This work
JP6795	JP6774 pJP819	This work
JP6796	JP6774 pJP820	This work
JP6797	JP6774 pJP821	This work
JP6798	JP6774 pJP822	This work
JP6799	JP6774 pJP823	This work
JP6800	JP6774 pJP824	This work
JP5332	JP4125 pJP661	This work
JP5333	JP4125 pJP766	This work
JP6833	JP4125 pJP813	This work
JP6834	JP4125 pJP814	This work
JP6835	JP4125 pJP815	This work
JP6836	JP4125 pJP817	This work
JP6837	JP4125 pJP818	This work
JP6838	JP4125 pJP819	This work
JP6839	JP4125 pJP820	This work
JP6840	JP4125 pJP821	This work
JP6841	JP4125 pJP822	This work
JP6842	JP4125 pJP823	This work
JP6843	JP4125 pJP824	This work

Plasmids	Description	Reference
pMAD	Vector for efficient allelic replacement	15
pCN51	Expression vector	9
pET28a	Expression vector	Novagen
pPROEX HTa	Expression vector	Invitrogen
pRN8298	Expression vector	9
pJP759	pMAD – Δdut φ11	This work
pJP760	pMAD – Δdut 80α	This work
pJP762	pMAD – Δsri 80α	This work
pJP763	pMAD – Δ Orf15 80α	This work
pJP653	pCN51 – <i>dut</i> φ11	This work
pJP658	pCN51 – <i>dut</i> PH15	This work
pJP751	pCN51 – Orf15 80α	This work
pJP752	pCN51 – Orf73 φ85	This work
pJP766	pCN51- <i>dut</i> φ11 Δcentral region	This work
pJP661	pCN51- <i>dut</i> PH15 central region φ11	This work
pJP919	pCN51- <i>dut</i> 80α	This work
pJP685	pCN51- <i>dut</i> 80α D95E	This work
pJP666	pET28a – <i>dut</i> φ11	This work
pJP667	pET28a – <i>dut</i> _{D81A} φ11	This work
pJP753	pET28a – <i>dut</i> 80α	This work
pJP754	pET28a - <i>dut</i> _{D95E} 80α	This work
pJP755	pET28a – <i>dut</i> φ11Δcentral region	This work
pJP675	pET28a – <i>dut</i> PH15	This work
pJP756	pPROEX HTa - His-Stl _{Sa} Pbov1 + <i>dut</i> φ11	This work
pJP757	pPROEX HTa - His-Stl _{Sa} Pbov1 + <i>dut</i> φPH15	This work
pJP758	pPROEX HTa - His-Orf15 + Stl _{Sa} Pbov2	This work
pJP811	pPROEX HTa - <i>dut</i> φ11	This work
pJP674	pRN8298-clor-pInt-20-19-18blaZ	This work
pJP813	pCN51-3xflag- <i>dut</i> φ11	This work
pJP814	pCN51-3xflag- <i>dut</i> φ11 D81A	This work
pJP815	pCN51-3xflag- <i>dut</i> φPH15	This work
pJP817	pCN51-3xflag- <i>dut</i> 80α K8R	This work
pJP818	pCN51-3xflag- <i>dut</i> 80α K8R, K12E	This work
pJP819	pCN51-3xflag- <i>dut</i> φ11 R8K, E12K	This work
pJP820	pCN51-3xflag- <i>dut</i> φ11 frameshift 3 rd codon	This work
pJP821	pCN51-3xflag- <i>dut</i> 80α	This work
pJP822	pCN51-3xflag- <i>dut</i> φ11 R8K	This work
pJP823	pCN51-3xflag- <i>dut</i> φ11 E12K	This work
pJP824	pCN51-3xflag- <i>dut</i> 80α K12E	This work

Supplementary Table 5 | Oligonucleotides used in this study.

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP653	Orf25phi11-4mS	ACGT <u>GTCGACGAAATTGAGAATAGCGTTGC</u>
	Orf25phi11-5cB	<u>CGCGGATCCCTTGACTCGATCTAAGATGTC</u>
pJP658	PH15-1mB	<u>CGCGGATCGAGAAATAGCGTTGCTACAGCTAGGGAGGA</u> GCAGGAAAATGACTAAAGAACTAGAAATTAAA
	PH15-4cE	<u>CCGGAATTCTGTATTCTTTAATCTCTGCTAC</u>
pJP666	Orf25phi11-8mB	<u>CGCGGATCCATGACTAACACATTACAAGTAAGG</u>
	Orf25phi11-9cS	<u>ACGCGTCGACCTTACACTCCGCTACTTCCG</u>
pJP667	Orf25phi11-8mB	<u>CGCGGATCCATGACTAACACATTACAAGTAAGG</u>
	Orf25phi11-6m	GAAACAGGCAAGATAGCTGCAGGATATCACGGC
	Orf25phi11-7c	GCCGTGATATCCTGCAGCTATCTGCCCTGTTTC
	Orf25phi11-9cS	<u>ACGCGTCGACCTTACACTCCGCTACTTCCG</u>
pJP675	PH15-19mB	<u>CGCGGATCCATGACTAAAGAACTAGAAATTAAA</u>
	PH15-4cE	<u>CCGGAATTCTGTATTCTTTAATCTCTGCTAC</u>
pJP751	Orf15-80α -7mB	<u>CGCGGATCCAATTAACTTAGTCATGAGATGG</u>
pJP752	Orf15-80α-8cK	<u>CGGGGTACCTTCAGTCATGTTCTACCTCC</u>
pJP753	Orf32phi80α -12mB	<u>CGCGGATCCATGACTAACACATTACAAGTAAAAC</u>
pJP754	Orf32phi80α -13cS	<u>ACGCGTCGACTCTTACACTCCGCTACTTCC</u>
pJP755	Orf25phi11-8mB	<u>CGCGGATCCATGACTAACACATTACAAGTAAGG</u>
	Orf25phi11-23c	TTTTTCTACTGTAATATCTTATCACCTATATTATCTGTGAT AATCCCTAAATTGCCGTGATATCC
	Orf25phi11-24m	ATCACAGATAATATAGGTGATAAAGATATTACAGTAGAAAA AGGAGACAAACTAGCTCAATTGGTT
	Orf25phi11-9cS	<u>ACGCGTCGACCTTACACTCCGCTACTTCCG</u>
pJP756	Orf25phi11-25mK	<u>CGGGGTACCTTCACACAGGAAACAGACCATGACTAACAC</u> ATTACAAGTAAGG
	Orf25phi11-26cH	<u>CCCAAGCTTCTGACTCGATCTAAGATGTC</u>
	SaPIbov1-162mB	<u>CGCGGATCCGATGGAAGGAGCTGGTCAAATGGC</u>
	SaPIbov1-150cK	<u>CGGGGTACCGATTAAATTAGTGTCTTTCAAG</u>
pJP757	PH15-8mK	<u>CGGGGTACCTTCACACAGGAAACAGACCATGACTAAAGA</u> ACTAGAAATTAAA
	PH15-9cH	<u>CCCAAGCTTGATTCTTTAATCTCTGCTAC</u>
	SaPIbov1-162mB	<u>CGCGGATCCGATGGAAGGAGCTGGTCAAATGGC</u>
	SaPIbov1-150cK	<u>CGGGGTACCGATTAAATTAGTGTCTTTCAAG</u>
pJP758	Orf15-phi80α-12mB	<u>CGCGGATCCGATGAAGCAGACTGTAACCTATC</u>
	Orf15phi80α-6cP	AACTGCAGTTCAGTCATGTTCTACCTCC
	SaPIbov2-orf22-5mP	AACTGCAGAATTCACACAGGAAACAGACCATGATTTATG TACATTAAAAATTATATG
	SaPIbov2-orf22-6cH	<u>CCCAAGCTTAAATAGTATGTAACCTTAAG</u>

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP759	Orf24-φ11-1mB	<u>CGCGGATCCTCAGGATAACGAACGAGTGGC</u>
	Orf25-φ11-1c	TTCTGATAATAGCCTTACTTG
	Orf25-φ11-2m	CAAGTAAGGCTATTATCAGAACCTACAGAAGCTGGAGAAAAAGGC
	Orf25-φ11-3cE	CCGGAATTCTTAACCACCACTAAACACCC
pJP760	Orf32-80α -3mB	CGCGGATCCATCGAGTTAAAGAAGGAGCC
	Orf32-80α -4c	CGTCTTATGATTCGTTGGG
	Orf32-80α -5m	CCCGAACGAAATCATAAGACGCCGAACTAAAGCAAGTGGAG
	Orf32-80α -6cS	ACGCGTCGACGCATCATTCTAACATAGCCC
pJP762	Orf22-80α -1mB	<u>CGCGGATCCAGCAATAGAGTACGTACAAGG</u>
	Orf22-80α -2c	GTACATATCTAACACTCAAG
	Orf22-80α -3m	CTTGAGTGTTCAGATATGTACCTGCAGAACGTCACACACG
	Orf21-80α -4cE	CCGGAATTCTCGACCATGATTAAAGTAATGG
pJP763	Orf15-80α -1mB	CGCGGATCCTTATCACCTCCTTCACTAGG
	Orf15-80α -2c	TACAGTCTGCTTCATAGTGAC
	Orf15-80α -3m	GTCACTATGAAGCAGACTGTAATGTCTAACGACAAAGCAATC
	Orf15-80α -4cE	CCGGAATTCTATTGTTCTCCTCACTATCC
pJP766	Orf25-φ11-4mS	ACGT <u>GTCGACGAAATTGAGAATAGCGTTGC</u>
	Orf25-φ11-23c	TTTTCTACTGTAATATCTTTATCACCTATATTCTGTGATAAT CCCTAAATTGCCGTGATATCC
	Orf25-φ11-24m	ATCACAGATAATATAGGTGATAAAAGATATTACAGTAGAAAAAG GAGACAAACTAGCTCAATTGGTT
	Orf25-φ11-5cB	<u>CGCGGATCCCTGACTCGATCTAACGATGTC</u>
pJP661	PH15-1mB	CGCGGATCCGAGAATAGCGTTGCTACAGCTAGGGAGGAGC AGGAAAATGACTAAAGAACTAGAAATTAAA
	PH15-2c	TGCAATAGCGTCATTCTGATATTAATACCGATTCTTATTGT ATG
	Orf25-φ11-22m	AATATCAAGAATGACGCTATTGCA
	PH15-4cE	<u>CCGGAATTGTATTCTTTAATCTCTGCTAC</u>
pJP674	SaPIbov1-149cB	<u>CGCGGATCCGATCAGTACCTAAATATGCG</u>
	NY-24mK	<u>CGGGGTACCCACTCGGTTATAACCTT</u>
pJP813	Orf25-φ11-35m	ATGGATTATAAGATCACGATGGCGATTATAAGATCACGATA TCGATTATAAGATGATGATGATAAAATGACTAACACATTACA AGTAAGG
	Orf25-φ11-5cB	<u>CGCGGATCCCTGACTCGATCTAACGATGTC</u>
	Orf32-80α -18mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAAATATGA GGAGGAATAGGAAAATGGATTATAAGATCACGATGG
	Orf25-φ11-35m	ATGGATTATAAGATCACGATGGCGATTATAAGATCACGATA TCGATTATAAGATGATGATGATAAAATGACTAACACATTACA AGTAAGG
pJP814	Orf25-φ11-5cB	<u>CGCGGATCCCTGACTCGATCTAACGATGTC</u>
	Orf32-80α -18mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAAATATGA GGAGGAATAGGAAAATGGATTATAAGATCACGATGG

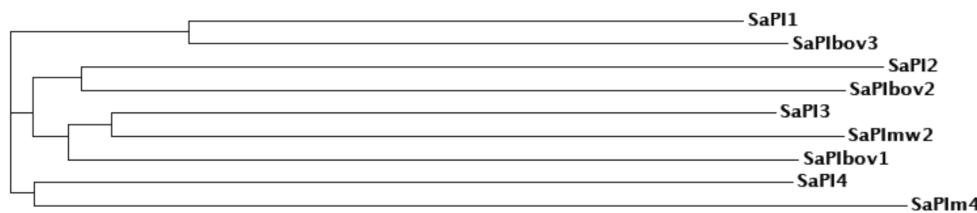
Plasmid	Oligonucleotides	Sequence (5'-3')
pJP815	PH15-10m	ATGGATTATAAAGATCACGATGGCGATTATAAAGATCAGC ATATCGATTATAAAGATGATGATGATAAAATGACTAAAGAA CTAGAAATTAAA
	PH15-11cB	<u>CGCGGATCCGTATTCTTTAATCTCTGCTAC</u>
	Orf32-80 α -18mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAATAT GAGGAGGAATAGGAAAATGGATTATAAAGATCACGATGG
pJP817	Orf32-80 α -24m	GATGGCGATTATAAAGATCACGATATCGATTATAAAGATG ATGATGATAAAATGACTAACACATTACAAGTAAGGCTATTA TCAAAAAATGCTAGAATGC
	Orf32-80 α -2cB	<u>CGCGGATCCTCACCAAAACCTCCTTGACTC</u>
	Orf32-80 α -23mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAATAT GAGGAGGAATAGGAAAATGGATTATAAAGATCACGATGG GATTATAAAGATCACG
pJP818	Orf32-80 α -22m	GATGGCGATTATAAAGATCACGATATCGATTATAAAGATG ATGATGATAAAATGACTAACACATTACAAGTAAGGCTATTA TCAGAAAATGCTAGAATGC
	Orf32-80 α -2cB	<u>CGCGGATCCTCACCAAAACCTCCTTGACTC</u>
	Orf32-80 α -23mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAATAT GAGGAGGAATAGGAAAATGGATTATAAAGATCACGATGGC GATTATAAAGATCACG
pJP819	Orf25- ϕ 11-43m	GATGGCGATTATAAAGATCACGATATCGATTATAAAGATG ATGATGATAAAATGACTAACACATTACAAGTAAACTATTA TCAAAAAATGCTAGAATGC
	Orf25- ϕ 11-5cB	<u>CGCGGATCCCTTGACTCGATCTAAGATGTC</u>
	Orf32-80 α -23mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAATAT GAGGAGGAATAGGAAAATGGATTATAAAGATCACGATGGC GATTATAAAGATCACG
pJP820	Orf25- ϕ 11-49m	ATGGATTATAAAGATCACGATGGCGATTATAAAGATCAGC ATATCGATTATAAAGATGATGATGATAAAATGACTACACAT TACAAGTAAGG
	Orf25- ϕ 11-5cB	<u>CGCGGATCCCTTGACTCGATCTAAGATGTC</u>
	Orf32-80 α -18mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAATAT GAGGAGGAATAGGAAAATGGATTATAAAGATCACGATGG
pJP821	Orf32-80 α -17m	ATGGATTATAAAGATCACGATGGCGATTATAAAGATCAGC ATATCGATTATAAAGATGATGATGATAAAATGACTAACACA TTACAAGTAAAC
	Orf32-80 α -2cB	<u>CGCGGATCCTCACCAAAACCTCCTTGACTC</u>
	Orf32-80 α -18mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAATAT GAGGAGGAATAGGAAAATGGATTATAAAGATCACGATGG
pJP822	Orf25- ϕ 11-44m	GATGGCGATTATAAAGATCACGATATCGATTATAAAGATG ATGATGATAAA ATGACTAACACATTACAAGTAAACTATTATCAGAAAATGC TAGAATGC
	Orf25- ϕ 11-5cB	<u>CGCGGATCCCTTGACTCGATCTAAGATGTC</u>
	Orf32-80 α -23mS	ACGCGTCGACATTATGGCAGGTCAAGTTGTCTATAAATAT GAGGAGGAATAGGAAAATGGATTATAAAGATCACGATGG CGATTATAAAGATCACG

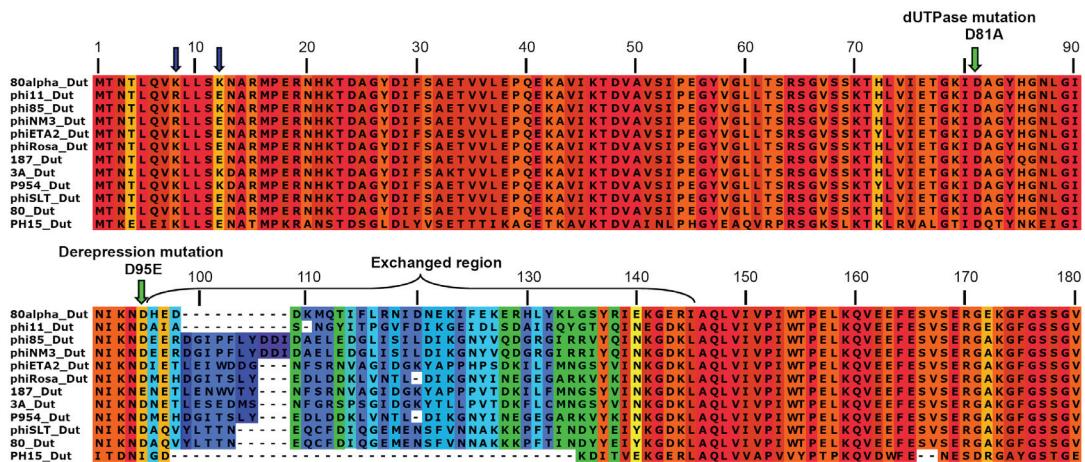
Plasmid	Oligonucleotides	Sequence (5'-3')
pJP823	Orf25- ϕ 11-45m	GATGGCGATTATAAAGATCACGATATCGATTATAAAGAT GATGATGATAAAATGACTAACACATTACAAGTAAGGCTA TTATCAAAAAATGCTAGAATGC
	Orf25- ϕ 11-5cB	<u>CGCGGATCC</u> CTTGACTCGATCTAAGATGTC
	Orf32-80 α -23mS	ACGCGT <u>CGAC</u> ATTATGGCAGGTCAAGTTGTCTATAAATA TGAGGAGGAATAGGAAAATGGATTATAAAGATCAC GATGGCGATTATAAAGATCACG
pJP824	Orf32-80 α -25m	GATGGCGATTATAAAGATCACGATATCGATTATAAAGAT GATGATGATAAAATGACTAACACATTACAAGTAAA TATCAGAAAATGCTAGAATGC
	Orf32-80 α -2cB	<u>CGCGGATC</u> CTCACCAAAACCTCCTTGACTC
	Orf32-80 α -23mS	ACGCGT <u>CGAC</u> ATTATGGCAGGTCAAGTTGTCTATAAATA TGAGGAGGAATAGGAAAATGGATTATAAAGATCAC GATGGCGATTATAAAGATCACG
Probe	Oligonucleotides	Sequence (5'-3')
EMSA	19-20upbov2	ATTTCAACATTAAACATTGC
	19-20dwbov	TAAATCCTGTCCTTCAC
SaPIbov1	SaPIbov1-112mE	<u>CCGGATT</u> CAATTGCTGAGGCAAAACTTC
	SaPIbov1-113cB	<u>CGCGGATC</u> CTAATTCTCCACGTCTAAAGC
SaPIbov2	Sip-16mB	<u>CGCGGATC</u> CCAATCCAATCAAACGCATGCG
	Sip-10cE	<u>CCGGATT</u> CTTCATGACTCTGTACGTT
ϕ11	Orf-24- ϕ 11-1mB	<u>CGCGGATC</u> CTCAGGATAACGAACGAGTGGC
	Orf25- ϕ 11-1c	TTCTGATAATAGCCTTACTTG

Sequences recognized by the restriction enzymes used in cloning are underlined.

A

SaPI1	-----MIYMTFGEILKKERVSWKLSVKELSTLGSVQTYISKLEN-GKRNFPSLETI	51
SaPIbov3	-----MKFSEMLKKYRTKENLSINKLAKLSGVSTTYISKLEK-NDRSYPTVEII	48
SaPI3	-----MRTNDEIITIITKTSMKEQNMSLSELARRVGVAKSARVSYLN-LTREFP-----	47
SaPImw2	-----MIIFRLKEIMEEKNLKISDLHEQTGISRNSISSLNGTRGIQ-----	43
SaPIbov1	MEGAGQMAELPHTHYGTIIKTLRKYMKLTQSLSERTGFSQNTISNHENGNRNIGVNEIEI	60
SaPI4	--MVESNDELKKELGRFLKSIRKQKGKTASEISKQMQYSQGHISGIEN-GVKSFPS---	53
SaPIbov2	-----MILCTLKNYMKLFGATQSQISEQTGITRPTLLSLIRNENKNIK-----	43
SaPI2	-----MIRNRLSELLSERGLKISRVAKDVKIARSSLTSMAQNDSEMIR-----	43
SaPIm4	-----MEEFGEKFTHKAHKSIIVSKWEGLTKPSNERLKEIAKLGNIJVHQLI-----	47
:		
SaPI1	FNLLIGFKTHIEYKMGSESPFYEINNSYLDEILIMFINSSNSTISDRDPNELITQFNEY	111
SaPIbov3	FNLAYGIIMKIKEKYDGIENSDDFLYPQIEEIISFFATSEDSNLDEENKNTIIDFIMFM	108
SaPI3	LNRAEDFAKVVLGIKT-----EYLLGFAEREESTKQDTIAAHLDGDFTEEEE	92
SaPImw2	FDTLEKITLALNVDVADLFKNVFNEIILIKLDDISKVETYRRSKFKKEKKNNIVKKYAVNC	103
SaPIbov1	YGKGLGIPSYLHLRISDEFKEKGYSPLNDFGKFDKMFMSYVNKAYYNDGDIYYSSYDLYD	120
SaPI4	NKLIESYLMNIKDTNEEYN-----FYVDEIAKTKNVKLNKVSNTNKMEIID	102
SaPIbov2	YDTIDELCNFFGSQLKDLLIYSPVKIKQKSFNICKTIEEYEHINESWKTGYVSIAYEINN	103
SaPI2	YDAIDKLCSYLHISPSEFFEHNPNINFDFDFTFDEEPNYKINDVFEFGFEVTAN-ITHAFSIEN	102
SaPIm4	YGDFLGLLESIANEEIK-----FILDTNMCANNFLANELSSSVSRFIFSY	93
:		
SaPI1	DVTI-KKKQNENSKIESDIFSNKIKLVKGTTKKEVIEKPYFDLNWLTTQNEYEVFFDRSF	170
SaPIbov3	ERKE-KEFLNKSFGDNKEIYENKIALVNSNMNYKTDYPYFDLKWLLSQNNFEVYGRDF	167
SaPI3	LIEI-RKY-----AELVRKAHRNQ-----	110
SaPImw2	DLIEDNDLKKGFIPYEISIELNPNPPEIEIKIQFDYSNLNFNYLIKFLDCNNFKLLLNVYL	163
SaPIbov1	ETIKLLELLKESKINVNNDIDYDVVLKLYQILSTDTEKSIINYETLANTRKSSDKKREVT	180
SaPI4	RMM-----IPYSREFISFDNNNEKSFTIFNISIN--DLHFHLQD	140
SaPIbov2	EDFIFEGS-----IDPIDLKTFKNKKFENGTLYLNNCNCFIEKDNYENLLKAGF	151
SaPI2	FDFEILVD-----VELDNRQKLNFDLDVSYKETEKITSQHRFIFTIKNE	147
SaPIm4	YERG-----KENFENENLFRKLLQHYLQLELDLGNRD	124
:		
SaPI1	LLDNNFLNKKHFTEKDM--YYYNVLNDNLKTIKDLIVVFLNKNYIKNKDDFFNIFTN	228
SaPIbov3	ITNFATIEDSKLNTKSM--YFYNILDKEDLKTIQRLIEVYLESKYPKIKDKDDFFVLATD	225
SaPI3	-----	
SaPImw2	SKKIYCLENKRINEIKS--FYS--IPDEKVYILSSFPGIFIR--RPLRDNNNGIFENIELN	217
SaPIbov1	IEEIGEFHEKYLKLLFTN-LETHNDRKKALAEIEKLKEESITYLGEKLRVLPNHHYDAIKG	239
SaPI4	INNYKFKYKGIR-----LTNDKNNIDKILNNYFENKSVIKENTKTLRDKNE	187
SaPIbov2	SKEFFDLYNDLN-----QIKNKIVDKLPFELDSLILLFNIFFNVRNAPSLEEYK	200
SaPI2	DENIGLKKYVDSLSSAGLKNLLFKKINQKLSGYVSEIIIVKNIDDEELFPNKGEKSTTLHK	207
SaPIm4	LESLTYFAYQR-----TINAQELVVDDYEDSKAKEFLKDESIDEFLTTISN	170
:		
SaPI1	SEDDKTKRDALYKILYETD-----	247
SaPIbov3	KQNRIKNTIDWYNIN-----	240
SaPI3	-----	
SaPImw2	KIINELNFSNNSNYNTYSDQITLTHKNKK	245
SaPIbov1	KPMYKLYLYEYPDRLEHQKKIILEKDTN	267
SaPI4	NWEQLVKLSDYIDDKLDKKN-----	207
SaPIbov2	EELQFLPTDSLVDLKNEIDKYLK-----	223
SaPI2	EILQTDSSRDLSSDIFKEY-----	224
SaPIm4	KYFDLLEYIDDYRVKHDLERKISEE-----	194

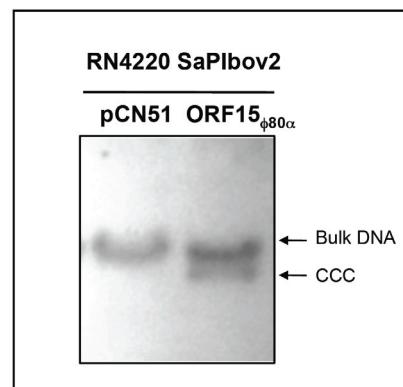
B



Supplementary Figure 2 | Alignment of predicted staphylococcal phage dUTPase protein sequences.

Colours indicate relative sequence conservation at each position, with red being most conserved and violet being least (adapted from alignment generated by PRALINE²¹).

Blue arrows indicate the two N-terminal variations between 80 α and ϕ 11. Green arrows indicate sites (81D and 95D) of mutations differentially affecting dUTPase activity and derepression, respectively. The bracket indicates the region that was exchanged between 80 α and ϕ 11 dUTPases.



Supplementary Figure 3 | De-repression of SaPIbov2 replication by cloned ORF15.

Southern blot of lysates from a non-lysogenic derivative of RN4220 carrying SaPIbov2 and either vector alone (pCN51, lane 1; strain JP6294) or plasmid expressing ORF15_{φ80α} (lane 2; strain JP6287). Cultures were grown until OD₅₄₀=0.3 and 1 ml samples were collected and used to prepare standard minilysates, which were resolved on a 0.7% agarose gel, Southern blotted, and probed for SaPIbov2 *int*. CCC, covalently closed circular SaPIbov2, which migrates near to the bulk DNA as a consequence of its relatively large size (~28 kb).

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ORF15 MKQTVTYLIKHDENLFITNRPTEVNDTVKYSTDMRDAREFDGLDKTVIDMSKHKAIKKT 60
ORF73 MNQTVTYIIRHRDMPYITNKPTDNNSDVSYSTNRNRAREFNGMEEASINMDYHKAIKKT 60
*:*****:*:*: * :****:***: *. *.*.*: . ****:*:***: *:*. *****

ORF15 VTETIEYEEVEHD 73
ORF73 VTETIEYEEVEHD 73
*****
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Supplementary Figure 4 | Comparison of two SaPlbov2 inducer proteins: ϕ 80 α ORF15 and ϕ 85 ORF73.

References

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