Supporting Information

Staphylococcal pathogenicty island DNA packaging system involving *cos*-site packaging and phage-encoded HNH endonucleases

Nuria Quiles-Puchalt, Nuria Carpena, Juan C. Alonso, Richard P. Novick, Alberto Marina, José R. Penadés.

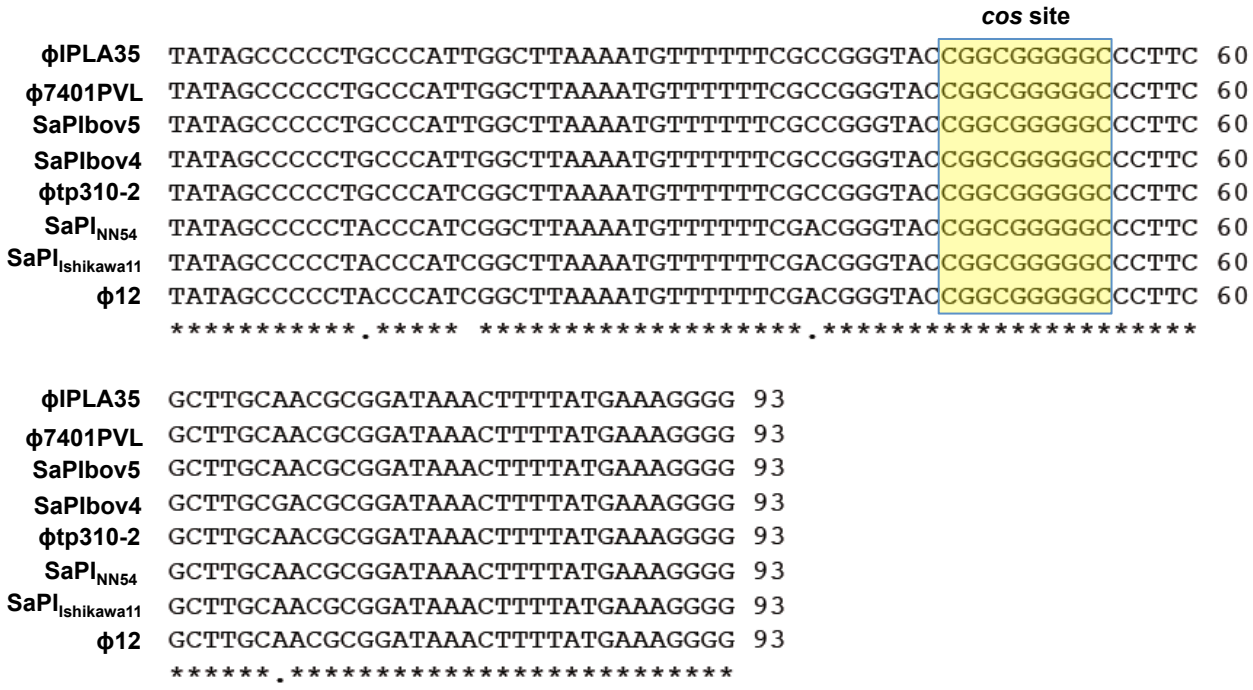
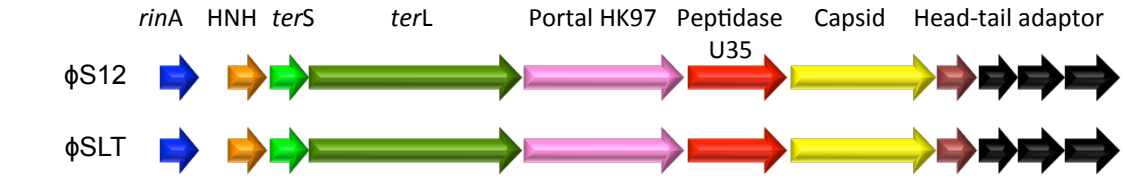


Figure S1. Cos alignment. The predicted SaPI and phage *cos* sites and their flanking sequences are aligned using ClustalW2. The *cos* sites are shaded in yellow.

Gram-positive



Gram-negative

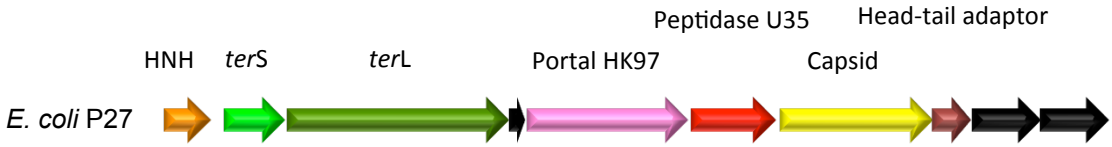


Figure S2. Alignment of selected genes from phage genomes coding for HNH proteins. Genes are coloured according to their sequence and function: *rin*A: blue; *hnh*: orange; *ter*S: light green; *ter*L: green; portal: pink; peptidase: red; capsid: yellow; head-tail adaptors: brown; hypothetical proteins: black.

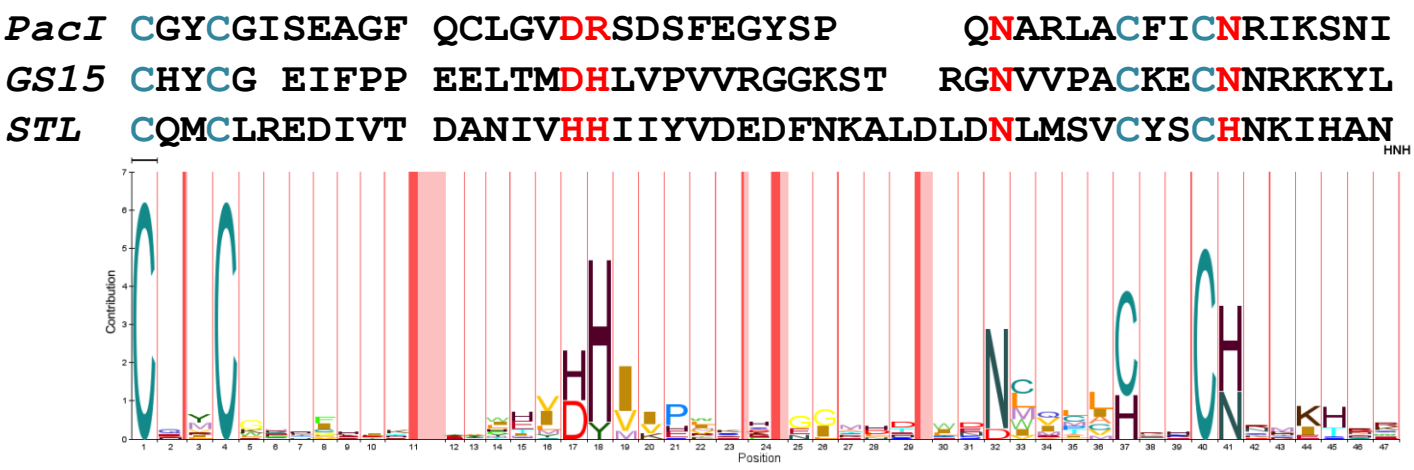


Figure S3. Sequence conservation in HNHs catalytic domains. HMM logo representation of PFAM family HNH (PF01844) (http://pfam.sanger.ac.uk/family/PF01844.18) generated by the alignment of 7400 sequences. Each position in the sequence is represented by a stack. The overall height of the stack indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino at that position. The sequence corresponding to the HNH domains of ϕSLT HNH (STL), GS-15 (GS15) and *Pac*I are aligned with the HHM logo and the catalytic and Zn-chelating residues displayed as stick in Fig. 3 are highlighted in red and cyan, respectively.

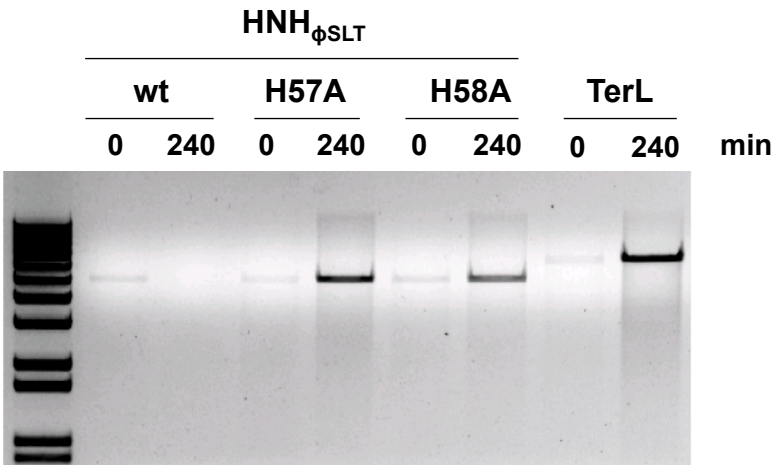


Figure S4. Plasmid DNA from *E. coli* strains expressing the different proteins was extracted and digested with *Bam*HI, which cuts once in the plasmid. Note that absence of plasmid in the sample obtained 240 min after IPTG induction of the wt HNH protein, which is indicative of unspecific nuclease activity. By contrast, neither the HNH mutants nor the TerL proteins showed nuclease activity.

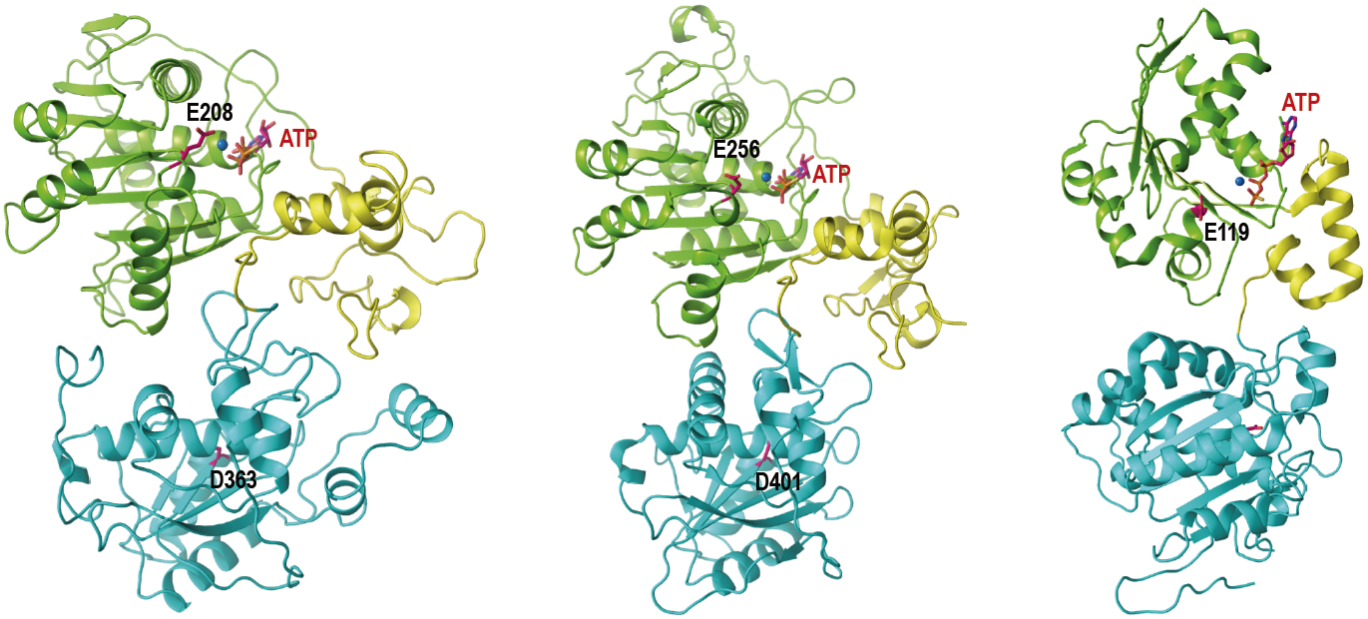
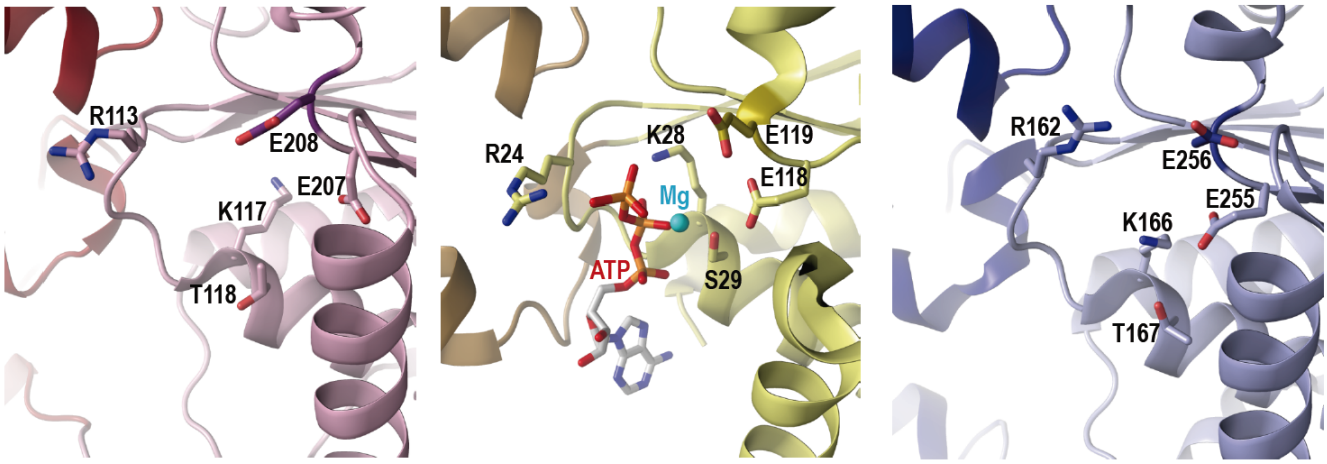


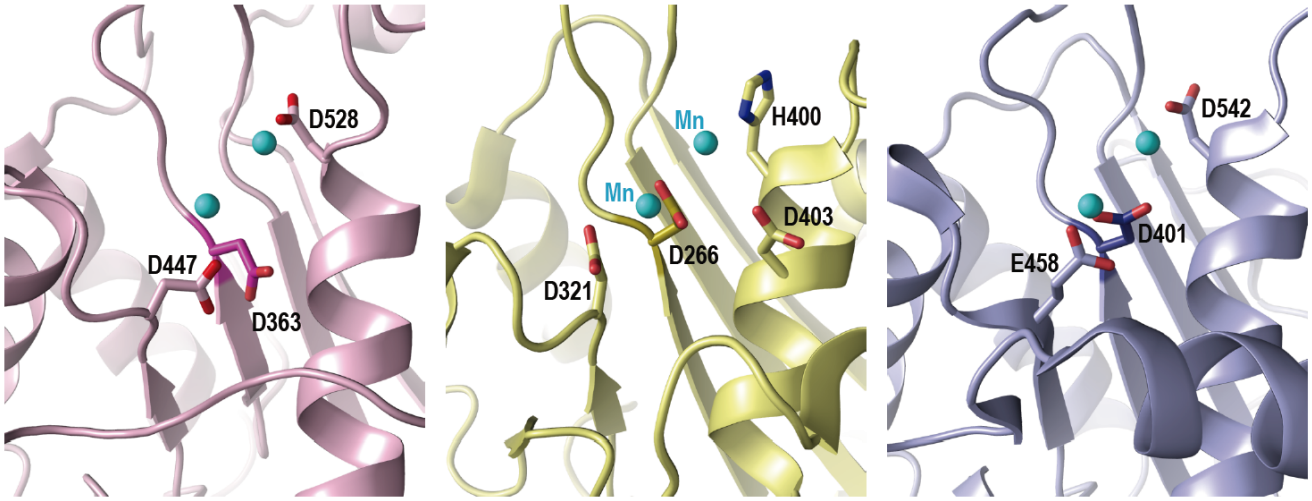
Figure S5. Overall modeled three-dimesional structure of $TerL_{\phi SLT.}$ The three-dimensional structure of $TerL_{\phi SLT}$ modeled with I-TASSER is shown in ribbon representation with the motor N-terminal domain colored in yellow (subdomain I) and green (subdomain II) and the nuclease C-terminal domain in cyan. $TerL_{\phi SLT}$ showed an overall fold that closely resembles to phage T4 gp17 (PDB 3CPE) and distally to phage Sf6 gp2 (PDB 4IEE) large terminases, which structures are shown in ribbon representation a colored as $TerL_{\phi SLT}$. Catalytic residues mutated $TerL_{\phi SLT}$ and the corresponding in T4 gp17 and Sf6 gp2 are shown in stick, labeled and colored in magenta. The ATP molecule present in the Sf6 gp2 structure was placed in $TerL_{\phi SLT}$ and T4 gp17 structures by superimposition of the corresponding motor domains and are shown in sticks with the carbon atom in magenta.

ATPase motif



ϕSLT **Sf6 gp2** **T4 gp17**

Nuclease



ϕSLT **SPP1** **T4 gp17**

Figure S6. ATPase and nuclease active sites of $TerL_{\phi SLT.}$ Close view of the ATPase (upper) and nucelase (lower) active sites of $TerL_{\phi SLT}$, Sf6 gp2 and T4 gp17 with catalytically important residues are shown as sticks. ATP molecule found in the ATPase site of Sf6 gp2 is shown in sticks with cabom atom in white. Cations found in Sf6 gp2 structure or modeled in the nuclease site of $TerL_{\phi SLT}$ and T4 gp17 structures are shown as cyan spheres. In $TerL_{\phi SLT}$ active site, the catalytic residues mutated in this work, Glu208 and Asp363, are highlighted in darker hues. Glu208 in the ATPase active site would correspond to the general base that polarises a water molecule for ATP nucleophilic attack. Asp363 is one of the three acidic residues forming the catalytic triad.

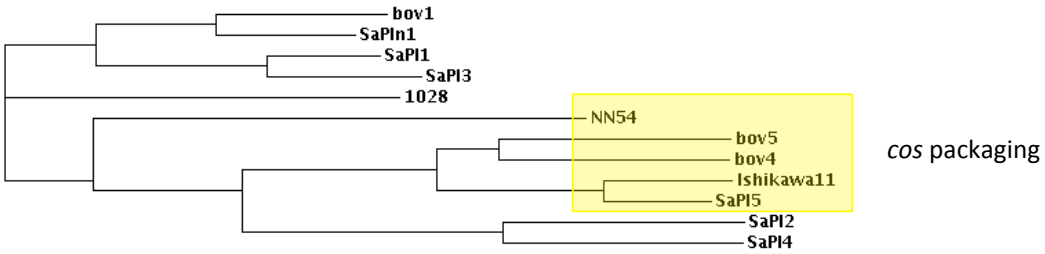


Figure S7. Bootstrapped Neighbor-Joining tree of different SaPIs. The tree was generated using ClustalW2 version. Shaded in yellow are the *cos*-type SaPIs.

Table S1. Role of the different cloned *cos* sites in pCU1 transfer[a].

Donor strain	Phage	Cloned site *cos*[b]	Plasmid titre[c]
JP10974	ϕ12	Empty vector	< 10
JP10968	ϕ12	ϕ12	2×10^3
JP10878	ϕ12	ϕ12 Δ*cos*	< 10
JP10907	ϕ12	ϕSLT	6×10^2
JP10879	ϕ12	ϕSLT Δ*cos*	< 10
JP10875	ϕ12	SaPIbov5	1×10^3
JP10876	ϕ12	SaPIbov5 Δ*cos*	< 10
JP10908	ϕ12	ϕP27[d]	< 10
JP10880	ϕ12	ϕP27 Δ*cos*	< 10
JP11204	ϕ11	Empty vector	6.2×10^3
JP11196	ϕ11	ϕ12	5×10^3
JP11197	ϕ11	ϕ12 Δ*cos*	9.6×10^3
JP11198	ϕ11	ϕSLT	2.4×10^3
JP11199	ϕ11	ϕSLT Δ*cos*	9.6×10^3
JP11200	ϕ11	SaPIbov5	3.1×10^3
JP11201	ϕ11	SaPIbov5 Δ*cos*	7×10^3
JP11202	ϕ11	ϕP27[d]	2.1×10^3
JP11203	ϕ11	ϕP27 Δ*cos*	1.1×10^3

[a]The means of results from three independent experiments are shown. Variation was within ±5% in all cases.
[b]In addition to the *cos* site, the cloned DNA contains ~50 pb of the flanking region to the *cos* site. The fragment cloned in the Δ*cos* plasmids are mutant in the *cos* site (maintaining the flaking sequences).
[c]No. of transductants/ml induced culture, using RN4220 as recipient strain.
[d]ϕP27 is an *E. coli* phage carrying a completely different *cos* site.

Table S2. Effect of ϕSLT mutations on phage titre[a].

ϕSLT	Complemented[b]	Phage transductants[c]
Wild type	Empty vector	5.0×10^5
Δ*hnh*		< 10
Δ*hnh*	pCN51-$hnh_{\phi SLT}$	1.9×10^3
HNH H57A		< 10
HNH H57A	pCN51-$hnh_{\phi SLT}$	2.2×10^2
HNH H58A		< 10
HNH H58A	pCN51-$hnh_{\phi SLT}$	1.3×10^2
Δ*ter*S		< 10
Δ*ter*S	pCN51-$terS_{\phi SLT}$	1.6×10^6
Δ*ter*L		< 10
Δ*ter*L	pCN51-$terL_{\phi SLT}$	4.6×10^5
TerL E208A		< 10
TerL E208A	pCN51-$terL_{\phi SLT}$	1.0×10^4
TerL D363A		<10
TerL D363A	pCN51-$terL_{\phi SLT}$	1.1×10^4
ΔPortal		< 10
ΔPortal	pCN51-$p40_{\phi SLT}$	1.2×10^5
Δ Prohead protease		< 10
Δ Prohead protease	pCN51-$p41_{\phi SLT}$	2.4×10^4
Δ Major capsid protein		< 10
Δ Major capsid protein	pCN51- $p42_{\phi SLT}$	1.4×10^5
Tail protein		< 10
Tail protein	pCN51- $p47_{\phi SLT}$	2.8×10^5

[a]The means of results from three independent experiments are shown. Variation was within ±5% in all cases.

[b]Complemented in donor strain.

[c]No. of transductants/ml induced culture, using RN4220 as recipient strain.

Table S3. Effect of ϕP27 mutations on phage titre[a].

ϕP27	Mutant in	Complemented[b]	Transductant titre[c]
Wild type		Empty vector	1.8×10^8
ΔORF34	*hnh*		< 10
ΔORF34	*hnh*	pBAD18-$hnh_{\phi P27}$	1.6×10^7
ΔORF35	*ter*S		< 10
ΔORF35	*ter*S	pBAD18-$terS_{\phi P27}$	1.5×10^5
ΔORF36	*ter*L		< 10
ΔORF36	*ter*L	pBAD18-$terL_{\phi P27}$	4.7×10^6
ΔORF38	Portal		< 10
ΔORF38	Portal	pBAD18-$p38_{\phi P27}$	2.8×10^3
ΔORF39	Prohead protease		< 10
ΔORF39	Prohead protease	pBAD18-$p39_{\phi P27}$	2.2×10^5
ΔORF40	Major capsid		< 10
ΔORF40	Major capsid	pBAD18-$p40_{\phi P27}$	5.5×10^6
ΔORF47	Tail protein		< 10
ΔORF47	Tail protein	pBAD18-$p47_{\phi P27}$	1.2×10^6

[a]The means of results from three independent experiments are shown. Variation was within ±5% in all cases.
[b]Complemented in donor strain.
[c]No. of transductants/ml induced culture, using MG1655 as recipient strain.

Table S4. Strains used in this study.

Strains	Description	Reference
RN4220	Restriction-defective derivative of RN450	(1)
RN451	RN450 lysogenic for ϕ11	(2)
RN10359	RN450 lysogenic for 80α	(3)
JP10435	RN4220 lysogenic for ϕ12	This work
JP1794	RN451 SaPIbov1 *tst*::*tet*M	(4)
JP7085	RN451 SaPIbov5 *tet*M	This work
JP3603	RN10359 SaPIbov1 *tst*::*tet*M	(4)
JP7084	RN10359 SaPIbov5 *tet*M	This work
JP11041	JP10435 SaPIbov1 *tst*::*tet*M	This work
JP11010	JP10435 SaPIbov5 *tet*M	This work
JP11215	RN4220 SaPIbov5 *tet*M Δ*cos* site	This work
JP11229	JP10435 SaPIbov5 *tet*M Δ*cos* site	This work
JP11228	RN451 SaPIbov5 *tet*M Δ*cos* site	This work
JP3377	RN451 Δ*ter*S	(4)
JP3378	JP3377 SaPIbov1 *tst*::*tet*M	(4)
JP10764	JP3377 SaPIbov5 *tet*M	This work
JP11401	JP10764 pJP1570	This work
JP10971	JP10435 Δ*hnh*	This work
JP11011	JP10971 SaPIbov5 *tet*M	This work
JP11406	JP11011 pJP1514	This work
LUG1170	SH1000 ϕSLT	(5)
JP11195	LUG1170 SaPIbov1 *tst*::*tet*M	This work
JP11194	LUG1170 SaPIbov5 *tet*M	This work
JP11230	LUG1170 SaPIbov5 *tet*M Δ*cos* site	This work
JP11402	LUG1170 ϕSLT Δ*hnh*	This work
JP11403	JP11402 SaPIbov5 *tet*M	This work
JP11404	JP11402 pJP1077	This work
JP11405	JP11403 pJP1077	This work
JP10817	RN4220 pJP1523	This work
JP10818	RN4220 pJP1524	This work
JP6847	RN4220 pJP836	This work
JP6848	RN4220 pJP837	This work
JP5011	RN4220 lysogenic for ϕSLT *pvl*::*tet*M	(6)
JP8240	JP5011 ϕSLT Δ*hnh*	This work
JP9104	JP5011 ϕSLT Δ*ter*S	This work
JP9105	JP5011 ϕSLT Δ*ter*L	This work
JP9106	JP5011 ϕSLT ΔORF40	This work
JP9107	JP5011 ϕSLT ΔORF41	This work
JP9108	JP5011 ϕSLT ΔORF42	This work
JP9951	JP5011 ϕSLT ΔORF47	This work
JP9666	JP5011 ϕSLT HNH H57A	This work
JP9667	JP5011 ϕSLT HNH H58A	This work
JP10934	JP5011 ϕSLT TerL E208A	This work
JP10976	JP5011 ϕSLT TerL D363A	This work
JP11367	JP5011 pCN51	This work
JP9059	JP8240 pJP1077	This work
JP9119	JP9104 pJP1245	This work

Strains	Description	Reference
JP9120	JP9105 pJP1246	This work
JP9121	JP9106 pJP1247	This work
JP9122	JP9107 pJP1248	This work
JP9123	JP9108 pJP1249	This work
JP10024	JP9951 pJP1520	This work
JP9959	JP9666 pJP1077	This work
JP9960	JP9667 pJP1077	This work
JP11307	JP10934 pJP1246	This work
JP11308	JP10976 pJP1246	This work
JP11074	JP10971 pJP1514	This work
JP11204	RN451 pCU1	This work
JP11198	RN451 pJP1525	This work
JP11199	RN451 pJP1526	This work
JP11196	RN451 pJP1527	This work
JP11197	RN451 pJP1528	This work
JP11200	RN451 pJP1529	This work
JP11201	RN451 pJP1530	This work
JP11202	RN451 pJP1531	This work
JP11203	RN451 pJP1532	This work
JP10974	JP10435 pCU1	This work
JP10907	JP10435 pJP1525	This work
JP10879	JP10435 pJP1526	This work
JP10968	JP10435 pJP1527	This work
JP10878	JP10435 pJP1528	This work
JP10875	JP10435 pJP1529	This work
JP10876	JP10435 pJP1530	This work
JP10908	JP10435 pJP1531	This work
JP10880	JP10435 pJP1532	This work
JP9818	STEC strain 2771/97 ϕP27	(7)
JP10045	JP9818 ϕP27 *stx*::*tet*A	This work
JP10363	*E. coli* strain MG1655	Lab strain
JP10819	JP10363 ϕP27 *stx*::*tet*A	This work
JP10960	JP10819 ϕP27 ΔORF34	This work
JP10961	JP10819 ϕP27 ΔORF35	This work
JP10962	JP10819 ϕP27 ΔORF36	This work
JP10963	JP10819 ϕP27 ΔORF38	This work
JP10964	JP10819 ϕP27 ΔORF39	This work
JP10965	JP10819 ϕP27 ΔORF40	This work
JP10967	JP10819 ϕP27 ΔORF47	This work
JP11327	JP10819 pBAD18	This work
JP11328	JP10960 pJP1539	This work
JP11329	JP10961 pJP1540	This work
JP11330	JP10962 pJP1541	This work

Strains	Description	Reference
JP11331	JP10963 pJP1542	This work
JP11332	JP10964 pJP1543	This work
JP11333	JP10965 pJP1544	This work
JP11334	JP10967 pJP1545	This work
JP10025	BL21 (DE3) pJP1533	This work
JP10566	BL21 (DE3) pJP1534	This work
JP10567	BL21 (DE3) pJP1535	This work
JP10317	BL21 (DE3) pJP1253	This work

Table S5. Plasmids used in this study.

Plasmid	Description	Reference
pMAD	Vector for efficient allelic replacement	(8)
pJP1510	pMAD derivative, deletion of ϕ12 *hnh*	This work
pJP1076	pMAD derivative, deletion of ϕSLT ORF 37	This work
pJP1240	pMAD derivative, deletion of ϕSLT ORF 38	This work
pJP1241	pMAD derivative, deletion of ϕSLT ORF 39	This work
pJP1242	pMAD derivative, deletion of ϕSLT ORF 40	This work
pJP1243	pMAD derivative, deletion of ϕSLT ORF 41	This work
pJP1244	pMAD derivative, deletion of ϕSLT ORF 42	This work
pJP1509	pMAD derivative, deletion of ϕSLT ORF 47	This work
pJP1254	pMAD derivative, mutation H57A in ϕSLT ORF 37	This work
pJP1255	pMAD derivative, mutation H58A in ϕSLT ORF37	This work
pJP1512	pMAD derivative, mutation E208A in ϕSLT ORF 39	This work
pJP1513	pMAD derivative, mutation D363A in ϕSLT ORF39	This work
pJP730	pMAD derivative, insertion of the *tetM* cassette into SaPIbov5	(9)
pJP1557	pMAD derivate, deletion *cos* site SaPIbov5	This work
pCN51	Expression vector	(10)
pJP1514	pCN51-*hnh* ϕ12	This work
pJP1077	pCN51-*hnh* ϕSLT	This work
pJP1245	pCN51-*ter*S ϕSLT	This work
pJP1246	pCN51-*ter*L ϕSLT	This work
pJP1247	pCN51-p40 ϕSLT	This work
pJP1248	pCN51-p41 ϕSLT	This work
pJP1249	pCN51-p42 ϕSLT	This work
pJP1520	pCN51-p47 ϕSLT	This work
pJP1570	pCN51-*ter*S ϕ11	This work
pCN42	Used in transcriptional fusions to the staphylococcal β−lactamase *blaZ*. Contains the P*cad* promoter	(10)
pJP1523	Transcriptional analysis of ϕ12 *hnh* in presence of RinA, pCN42 derivate	This work
pJP1524	Transcriptional analysis of ϕ12 *hnh* in absence of RinA, pCN42 derivate	This work
pJP836	Transcriptional analysis of ϕSLT *hnh* in presence of RinA, pCN42 derivate	This work
pJP837	Transcriptional analysis of ϕSLT *hnh* in absence of RinA, pCN42 derivate	This work
pKD46	Plasmid with Red system of lambda phage.	(11)
pCP20	Plasmid for cassette replacement using FRTs in *E. coli*.	(11)
pBAD18	Expression vector	(12)
pJP1539	pBAD18-*hnh* ϕ27	This work
pJP1540	pBAD18-*ter*S ϕ27	This work
pJP1541	pBAD18- *ter*L ϕ27	This work
pJP1542	pBAD18-p38 ϕ27	This work
pJP1543	pBAD18-p39 ϕ27	This work
pJP1544	pBAD18-p40 ϕ27	This work
pJP1545	pBAD18-p47 ϕ27	This work
pCU1	Cm^r. Cloning vector	(13)
pJP1525	pCU1 *cos* site ϕSLT	This work
pJP1526	pCU1 Δ*cos* site ϕSLT	This work
pJP1527	pCU1 *cos* site ϕ12	This work
pJP1528	pCU1 Δ*cos* site ϕ12	This work
pJP1529	pCU1 *cos* site ϕSaPIbov5	This work
pJP1530	pCU1 Δ*cos* site ϕSaPIbov5	This work

Plasmid	Description	Reference
pJP1531	pCU1 *cos* site ϕP27	This work
pJP1532	pCU1 Δ*cos* site ϕP27	This work
pJP1533	Expression in *E. coli* of His-HNH ϕSLT, pPROEX HTa derivative	This work
pJP1534	Expression in *E. coli* of His-HNH H57A mutant ϕSLT, pPROEX HTa derivative	This work
pJP1535	Expression in *E. coli* of His-HNH H58A mutant ϕSLT , pPROEX HTa derivative	This work
pJP1253	Expression in *E. coli* of His-terL ϕSLT, pPROEX HTa derivative	This work

Table S6. Primers used in this study.

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP1510	phi12-1mB	CGCGGATCCGCATCATACGATATTAAGCCA
	phiSLTp37-1c	GTATTGATATGACTTACGACC
	phiSLTp37-2m	GGTCGTAAGTCATATCAATACCTAATGTCAGTTTGTTATAGC
	phiSLTp37-3cE	CCGGAATTCTTCATCAAATACCCATTTACC
pJP1076	phiSLT-p36-5mB	CGCGGATCCTTGCAGAATAAAGAACTAACG
	phiSLTp37-1c	GTATTGATATGACTTACGACC
	phiSLTp37-2m	GGTCGTAAGTCATATCAATACCTAATGTCAGTTTGTTATAGC
	phiSLTp37-3cE	CCGGAATTCTTCATCAAATACCCATTTACC
pJP1240	phiSLTp37-4mB	CGCGGATCCTAAGTAAACGAGGCACATCGC
	phiSLTp36-10c	TAACTTCATATAAAGACCCCC
	phiSLTp38-4m	GGGGGTCTTTATATGAAGTTACAAGAAGAAGGTGGTTTTGGT
	phiSLTp38-5cE	CCGGAATTCCAACTTATCACTGTCTGAAGC
pJP1241	phiSLTp37-4mB	CGCGGATCCTAAGTAAACGAGGCACATCGC
	phiSLTp39-1c	CATTTAAAACTTTAATAGTCACC
	phiSLTp39-2m	GGTGACTATTAAAGTTTTAAATGAGTGGTGAAGGAAACATAGAG
	phiSLTp39-3cE	CCGGAATTCCACATCAATCGGACTAATGCC
pJP1242	phiSLTp40-1mB	CGCGGATCCTTCGCTAATAACGACGAAATG
	phiSLTp40-2c	AATTTTTTCTTTATGCGTGTGAC
	phiSLTp40-3m	GTCACACGCATAAAGAAAAAATTTTATACCCAATTGACACGCCAC
	phiSLTp40-4cE	CCGGAATTCTTTAGGTGTTTCAACCAATTC
pJP1243	phiSLTp41-1mB	CGCGGATCCTTTGATAATGCAGTAAGAACC
	phiSLTp41-2c	CCTTTACTTTTTGATTTTCTTTTC
	phiSLTp41-3m	GAAAAGAAAATCAAAAAGTAAAGGGAAAAAATTAAACGCGAATGC
	phiSLTp41-4cE	CCGGAATTCTACTAAATCTACATCTGATCC
pJP1244	phiSLTp41-5mB	CGCGGATCCTTATACCCAATTGACACGCCAC
	phiSLTp42-1c	TTCATATAATGTCGGCATTTC
	phiSLTp42-2m	GAAATGCCGACATTATATGAAGATCAGCAACGTACATTAGAC
	phiSLTp42-3cE	CCGGAATTCTAAAGCTCTATCACTCTTAGC
pJP1509	phiSLTp47-1mB	CGCGGATCCTTTAAAGATACGGGTGCTAGC
	phiSLTp47-2c	ATAAGAACCTTGTCCTTCTGC
	phiSLTp47-3m	CAGAAGGACAAGGTTCTTATGACAGTGAAGATCATTCAGAG
	phiSLTp47-4cE	CCGGAATTCTGCTTCGCCCATTGTCACATC
pJP1254	phiSLT-p36-5mB	CGCGGATCCTTGCAGAATAAAGAACTAACG
	phiSLTp37-15c	AAAATCTTCGTCGACATAAATAATGTGTGCTACAATGTTTGCATC
	phiSLTp37-14m	GATGCAAACATTGTAGCACACATTATTTATGTCGACGAAGATTTT
	phiSLTp37-3cE	CCGGAATTCTTCATCAAATACCCATTTACC
pJP1255	phiSLT-p36-5mB	CGCGGATCCTTGCAGAATAAAGAACTAACG
	phiSLTp37-17c	AAAATCTTCGTCGACATAAATAATTGCATGTACAATGTTTGCATC
	phiSLTp37-16m	GATGCAAACATTGTACATGCAATTATTTATGTCGACGAAGATTTT
	phiSLTp37-3cE	CCGGAATTCTTCATCAAATACCCATTTACC

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP1512	phiSLTp39-26mS	ACGCGTCGACTCTATGGGTTTAACTGCAGCA
	phiSLTp39-23c	GAAATCAATTTATAATCTTTGAATTCATGAATTGCATCAAAAATACCCAT ATGTGTATTT
	phiSLTp39-22m	AAATACACATATGGGTATTTTTGATGCAATTCATGAATTCAAAGATTATA AATTGATTTC
	phiSLTp39-27cB	CGCGGATCCCTCTTTTGCGATAACATTCAC
pJP1513	phiSLTp39-4mB	CGCGGATCCTCTATGGGTTTAACTGCAGCA
	phiSLTp39-25c	TAAAGTCCTCTGTTTCTGATAATGCATAACCTATAGTACATGGTCGACC TTCCAACTCATCTAAGGAAAT
	phiSLTp39-24m	ATTTCCTTAGATGAGTTGGAAGGTCGACCATGTACTATAGGTTATGCAT TATCAGAAACAGAGGACTTTA
	phiSLTp39-5cE	CCGGAATTCCTCTTTTGCGATAACATTCAC
pJP1557	SaPIbov5-45mB	CGCGGATCCGAGGGACATATCTATACAGAG
	SaPIbov5-46c	CGCGTTGCAAGCGAAGGGTTTTTTTTTTTACCCGGCGAAAAAACATTTT AAGCCCATGGGCAGGGGGCTATATTTTTTAT
	SaPIbov5-47m	ATAAAAAATATAGCCCCCTGCCCATGGGCTTAAAATGTTTTTTCGCCG GGTAAAAAAAAAAACCCTTCGCTTGCAACGCG
	SaPIbov5-48cE	CCGGAATTCATCATCTCCGTCCCATTCACC
pJP1514	phi12p28-1mB	CGCGGATCCGGCACATCGCTATGCGGTGTG
	phi12p28-2cE	CCGGAATTCTAGGGGGCTATAAAAATAAATTA
pJP1077	phiSLTp37-4mB	CGCGGATCCTAAGTAAACGAGGCACATCGC
	phiSLTp36-4cE	CCGGAATTCAAACATTTTAAGCCGATGGGC
pJP1245	phiSLTp38-6mB	CGCGGATCCGAGAGGCCCAAACGCTAGC
	phiSLTp38-7cE	CCGGAATTCGTTGTTAATAGTTTTGGTGAAGG
pJP1246	phiSLTp39-4mB	CGCGGATCCTCTATGGGTTTAACTGCAGCA
	phiSLTp39-5cE	CCGGAATTCCTCTTTTGCGATAACATTCAC
pJP1247	phiSLTp40-5mB	CGCGGATCCAGTGGTGAAGGAAACATAGAG
	phiSLTp40-6cE	CCGGAATTCCCTTTACTTTTTGATTTTCTTTTC
pJP1248	phiSLTp41-5mB	CGCGGATCCTTATACCCAATTGACACGCCAC
	phiSLTp41-6cE	CCGGAATTCTTCATATAATGTCGGCATTTC
pJP1249	phiSLTp42-4mB	CGCGGATCCCCTAAAGAAAGTATGTCACTAG
	phiSLTp42-5cE	CCGGAATTCGCTGATTTAGCCTTAGCTG
pJP1520	phiSLTp47-5mS	ACGCGTCGACCAGCGCATCGAATAGGTGTG
	phiSLTp47-6cB	CGCGGATCCCTGCTACTTCAACATTTTGGG
pJP1570	orf29phi11-21mB	CGCGGATCCTGGGTTGGCTGATTATAGCC
	orf29phi11-22cE	CCGGAATTCGTTAAAGTTTAATTTAACTTTCG
pJP1523	phi12p27-1mS	ACGCGTCGACGGGCACATTATTGTTTGGT
	phi12p27-4cB	CGCGGATCCTCTTCACGTAAACACATTTGAC

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP1524	phi12p27-1mS	ACGCGTCGACGGGCACATTATTGTTTGGT
	phi12p27-2c	AGCTCTTTCGTTGGGTTAA
	phi12p27-3m	TTAACCCAACGAAAGAGCTGAACTTTGTTAAAGCGGTAG
	phi12p27-4cB	CGCGGATCCTCTTCACGTAAACACATTTGAC
pJP836	phiSLTp36-7mB	CGCGGATCCGACATTAAGTTGCTTATAGCG
	phiSLTp36-8cE	CCGGAATTCCTCTCTTAACTTCTTCCATGC
pJP837	phiSLTp36-7mB	CGCGGATCCGACATTAAGTTGCTTATAGCG
	phiSLTp36-2c	GATATCATATATTGTGTTCCC
	phiSLTp36-3m	GGAACACAATATATGATATCAACTTTGTTAAAGCGGTAGCG
	phiSLTp36-8cE	CCGGAATTCCTCTCTTAACTTCTTCCATGC
pJP1539	phiP27-HNH-15mXba1	GCTCTAGAAGTAACAGGCATTACAGCAGC
	phiP27-HNH-16cH	CCCAAGCTTTCACCTGATTCGTTCGCGCG
pJP1540	phiP27-terS-15mXba1	GCTCTAGAAATTTTTACACCCGCGAAATT
	phiP27-terS-16cH	CCCAAGCTTTCATAGGTTTTTAAATGGATTATCGC
pJP1541	phiP27-terL-15mXba1	GCTCTAGAGGGAGCGATAATCCATTTAAAA
	phiP27-terL-16cH	CCCAAGCTTTTAAAGCGAACGGATCCCGTA
pJP1542	phiP27p38-5mXba1	GCTCTAGAGCACGATATCTCGACCGTACA
	phiP27p38-6cH	CCCAAGCTTTCAAGACGCTGTTTTGTCTGC
pJP1543	phiP27p39-6mXba1	GCTCTAGAGATGAACATGACCACCAGTCC
	phiP27p39-5cH	CCCAAGCTTTTAAAATTTAAGATTTTTCAGTGCATTC
pJP1544	phiP27p40-5mXba1	GCTCTAGACGCACTGAATGCACTGAAAAA
	phiP27p40-6cH	CCCAAGCTTTTACGCAGCGGCTTTCTGG
pJP1545	phiP27p47-5mXba1	GCTCTAGATATTTCGCAGTTTACAGAACTG
	phiP27p47-6cH	CCCAAGCTTTTAACGCCTGAAAGGTGAATA
pJP1525	phiSLT-45mH	CCCAAGCTTTTTTATGCCCCCCTGCC
	phiSLT-46cB	CGCGGATCCGACCCCCTTTCATGAAAAAT
pJP1526	phiSLT-50mE	CCGGAATTCCCCCCCTGCCCATCGGCTTAAAATGTTTTTTCGCCGGGT AAAAAAAAAAACCAAACGCTAGCAACGCGGA
	phiSLT-51cB	CGCGGATCCCCCCTTTCATGAAAAATTTATCCGCGTTGCTAGCGTTTG GTTTTTTTTTTTACCCGGCGAAAAAACATTT
pJP1527	phi12-7mE	CCGGAATTCGCCCCCTACCCATCGGCTTAAAATGTTTTTTCGACGGGT ACCGGCGGGGGCCCTTCGCTTGCAACGCGGA
	phi12-8cB	CGCGGATCCCCCCCTTTCATAAAAGTTTATCCGCGTTGCAAGCGAAGG GCCCCCGCCGGTACCCGTCGAAAAAACATTT

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP1528	phi12-9mE	CCGGAATTCGCCCCCTACCCATCGGCTTAAAATGTTTTTTCGACGGGT AAAAAAAAAAACCCTTCGCTTGCAACGCGGA
	phi12-10mB	CGCGGATCCCCCCCTTTCATAAAAGTTTATCCGCGTTGCAAGCGAAGG GTTTTTTTTTTTACCCGTCGAAAAAACATTT
pJP1529	SaPIbov5-41mE	CCGGAATTCGCCCCCTGCCCATTGGCTTAAAATGTTTTTTCGCCGGGT ACCGGCGGGGGCCCTTCGCTTGCAACGCGGA
	SaPIbov5-42cB	CGCGGATCCCCCCCTTTCATAAAAGTTTATCCGCGTTGCAAGCGAAGG GCCCCCGCCGGTACCCGGCGAAAAAACATTT
pJP1530	SaPIbov5-43mE	CCGGAATTCGCCCCCTGCCCATTGGCTTAAAATGTTTTTTCGCCGGGT AAAAAAAAAAACCCTTCGCTTGCAACGCGGA
	SaPIbov5-44cB	CGCGGATCCCCCCCTTTCATAAAAGTTTATCCGCGTTGCAAGCGAAGG GTTTTTTTTTTTACCCGGCGAAAAAACATTT
pJP1531	phip27-18mE	CCGGAATTCAGGGGCGGGTCAAATCCCTGCAACCCTGGCTGTCCGG GACCGCCCGCCCCGTCAAATTTTTACACCCGCG
	phip27-19cB	CGCGGATCCAATCCTGAAATTTTTAATTTCGCGGGTGTAAAAATTTGAC GGGGCGGGCGGTCCCGGACAGCCAGGGTTG
pJP1532	phip27-16mE	CCGGAATTCAGGGGCGGGTCAAATCCCTGCAACCCTGGCTGTCCGG GACAAAAAAAAAAGTCAAATTTTTACACCCGCG
	phip27-17cB	CGCGGATCCAATCCTGAAATTTTTAATTTCGCGGGTGTAAAAATTTGAC TTTTTTTTTTGTCCCGGACAGCCAGGGTTG
pJP1533	phiSLTp37-9mB	CGCGGATCCGATGACCAAGCATAATAACATT
	phiSLTp37-10cE	CCGGAATTCTTAAATTTTTAGAACTCTAATTTTC
pJP1534	phiSLTp37-9mB	CGCGGATCCGATGACCAAGCATAATAACATT
	phiSLTp37-10cE	CCGGAATTCTTAAATTTTTAGAACTCTAATTTTC
pJP1535	phiSLTp37-9mB	CGCGGATCCGATGACCAAGCATAATAACATT
	phiSLTp37-10cE	CCGGAATTCTTAAATTTTTAGAACTCTAATTTTC
pJP1253	phiSLTp39-7mB	CGCGGATCCGGTGGTTTTGGTGACTATTAAAG
	phiSLTp39-8cE	CCGGAATTCTTAACGCATTATGTCTTTAATAC

*Underlined is shown the sequence recognized by the restriction enzymes used.

ϕP27 Mutagenesis	Oligonucleotides	Sequence (5'-3')
ϕP27 *stx*::*tet*A	phip27-1m	GGGTCTGGTACTGATTACCTTAGCCAAAAGGAATATATGTATATGAAG TGTATATTGTTACGCTGTTAACTACTTTACTT
	phip27-2c	TGCGTCCAGAAACAAAAGACGCGCATAAATAAACCGTAGATTCTCAGT TAAACTTCACCTGGTTATCAAGAGGGTCATTA
ϕP27 *stx*::*tet*A *hnh*::*clor*	phip27-HNH-1m	TTCAGTGAGGGGCTGCGATAATGCCGGTATTAAGGAGATTCCAATGC CATCACGAATACCTGTGTAGGCTGGAGCTGCTTC
	phip27-HNH-2c	ATTTGACCCGCCCCTCCCCACAAGTGAGAATAATTATCACCTGATTCG TTCGCGCGCTGTCATATGAATATCCTCCTTA
ϕP27 *stx*::*tet*A *ter*S::*clor*	phip27-terS-5m	CCGCGAAATTAAAAATTTCAGGATTTGACATGTCAGGAAAATCTGTTGC GCCCGGAAGAGTGTGTAGGCTGGAGCTGCTTC
	phip27-terS-6c	TTTTTTCGTGTCATAGGTTTTTAAATGGATTATCGCTCCCCTGCTTCGG CGTCATAAGCCCATATGAATATCCTCCTTA

ϕ**P27 Mutagenesis**	**Oligonucleotides**	**Sequence (5'-3')**
ϕP27 *stx*::*tet*A *ter*L::*clor*	phip27-terL-6m	CACGAAAAAATTACGTGAACGTTAACGCCGCAAATCAGTATGCACGTG ACGTGGTTCGCGTGTGTAGGCTGGAGCTGCTTC
	phip27-terL-7c	GCGCGAGAATCAGCATGATCATAATTACCTCAGTTAAAGCGAACGGAT CCCGTAGGACTCCATATGAATATCCTCCTTA
ϕP27 *stx*::*tet*A *orf38*::*clor*	phip27-p38-1m	ACACGGCAGTCTGTCGGCGGAGGTAAATAGTGTTCTTTTCGGGGTTAT TTCAACGAAAAATGTGTAGGCTGGAGCTGCTTC
	phip27-p38-2c	ACTGACGGATTTCAGGTTCAGCGGTATATCAAGACGCTGTTTTGTCTG CATCTCCACTCTCCATATGAATATCCTCCTTA
ϕP27 *stx*::*tet*A *orf39*::*clor*	phip27-p39-1m	TGACAACGGTAAGAAAAAGGAGAGTGGAGATGCAGACAAAACAGCGT CTTGATATACCGCTGTGTAGGCTGGAGCTGCTTC
	phip27-p39-2c	TCTTTAACATCAGCCATTATTTTCTCCTGGTTAAAATTTAAGATTTTTCA GTGCATTCAGCATATGAATATCCTCCTTA
ϕP27 *stx*::*tet*A *orf40*::*clor*	phip27-p40-1m	TGAAAAATCTTAAATTTTAACCAGGAGAAAATAATGGCTGATGTTAAAG ATGTGGAACAGTGTGTAGGCTGGAGCTGCTTC
	phip27-p40-2c	GCCCGCCATTACGCGGGCACAAAAAAAACCGCATTACGCAGCGGCTT TCTGGCGGGTTGCCATATGAATATCCTCCTTA
ϕP27 *stx*::*tet*A *orf47*::*clor*	phip27-p47-1m	GAACTGGAAGCCGAGGCCGTCCGCATTAACGAGGAGATGAAGCATGG CTGACAGTTTTCATGTGTAGGCTGGAGCTGCTTC
	phip27-p47-2c	AAGGTAAGGAAGGAAAATCCATTCTGATACCCCATTATTAACGCCTGA AAGGTGAATACCCATATGAATATCCTCCTTA

Southern blot	**Oligonucleotides**	**Sequence (5'-3')**
SaPIbov1 / SaPIbov5 probe	SaPIbov1-112mE	CCGGAATTCAATTGCTGAGGCAAAACTTC
	SaPIbov1-113cB	CGCGGATCCTAATTCTCCACGTCTAAAGC
ϕSLT probe (*rin*A)	phiSLTp36-1mB	CGCGGATCCATCAGAGCTGAAGTTTCATGG
	phiSLTp36-2c	GATATCATATATTGTGTTCCC
ϕSLT probe (*ter*S)	phiSLTp38-9mB	CGCGGATCCGATGAAAGGGGGTCTTTATATG
	phiSLTp37-5c	AATAAAATGTTGCCAAGGCTG
ϕP27 probe (*ter*S-*ter*L)	phip27-terL-2c	CGCGTTGGGATTACGGTAGAG
	phiP27-terS-7c	CACTCCCCTTTGGTGT

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