Science

Supplementary Materials for

Mobile integrons encode phage defense systems

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The PDF file includes:

Figs. S1 to S8
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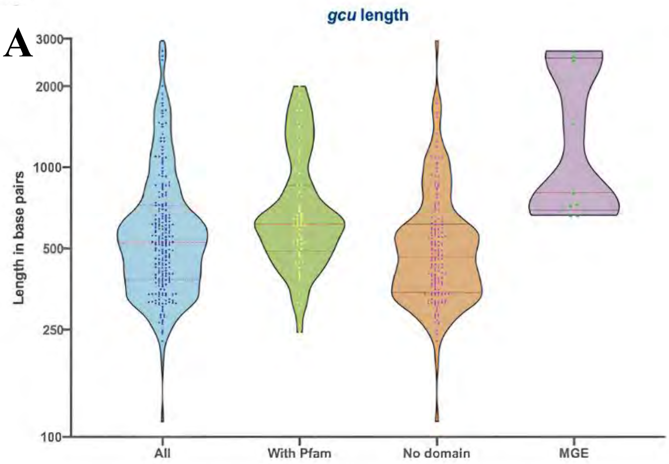
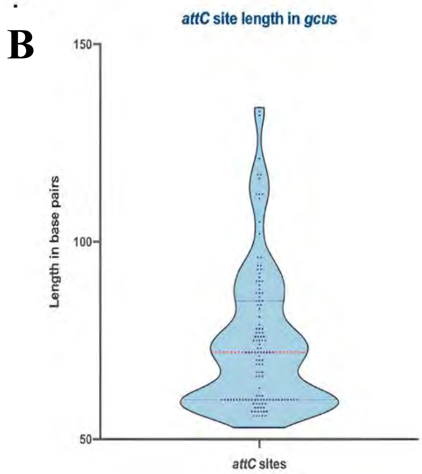
A



B



C

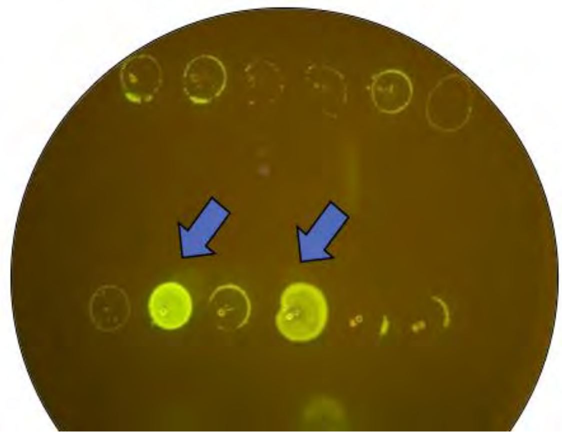


Fig. S1. Length distribution of *gcus* and *attC* Sites. **(A)** Violin plot depicting the length distribution of *gcus* in base pairs across various categories. **(B)** Violin plot showing the length distribution of *attC* sites in *gcus*, measured in base pairs. The *attC* sites are essential for site-specific recombination mediated by integrases. **(C) Double spot screening for phage resistance** Identification of *gcu24* and *gcu142* through double spot screening. The image shows the results of a phage resistance assay, where bacterial spots containing different *gcus* were challenged with phage T4. The arrows indicate the spots corresponding to *gcu24* and *gcu142*, which show growth despite the presence of the phage, indicating successful identification and resistance.

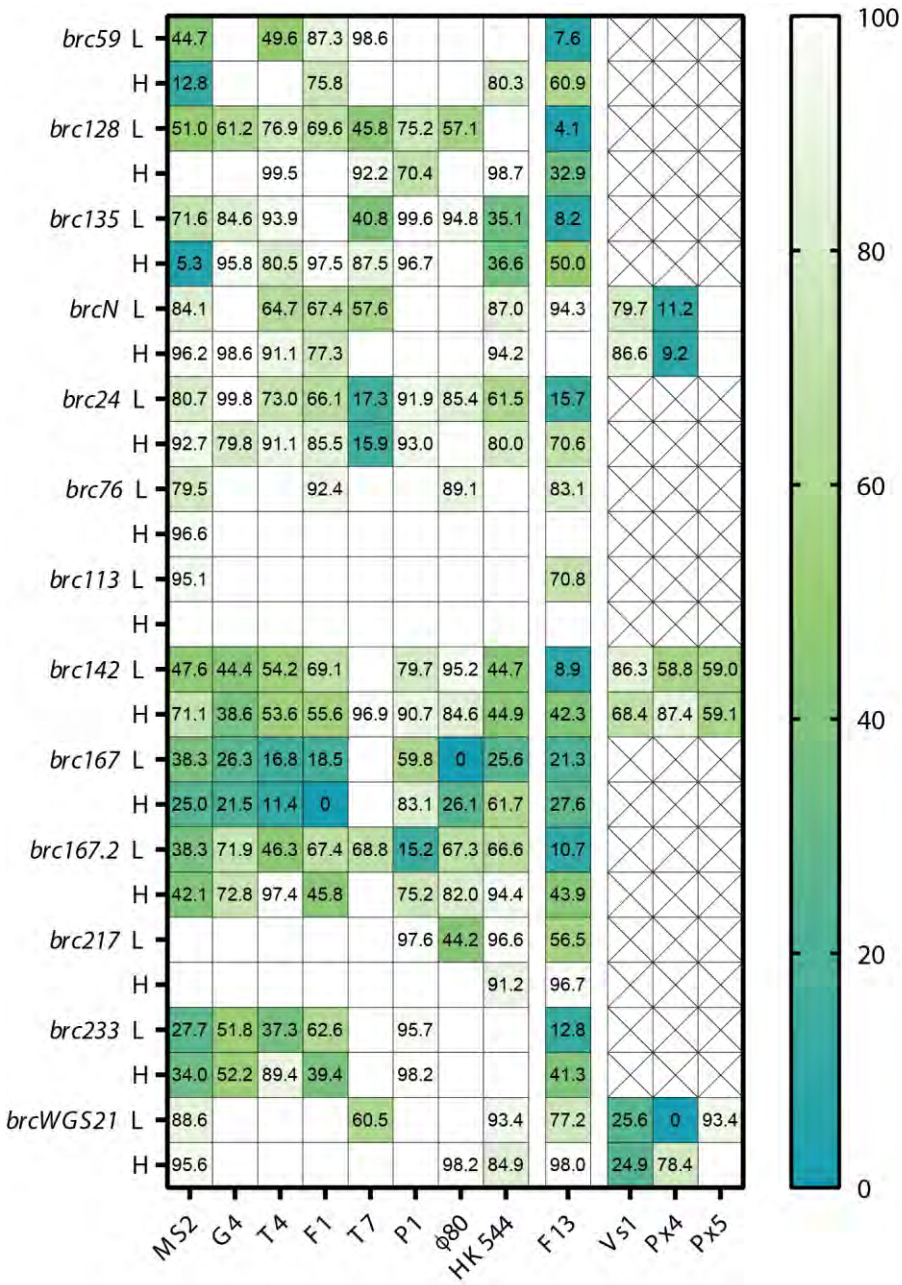
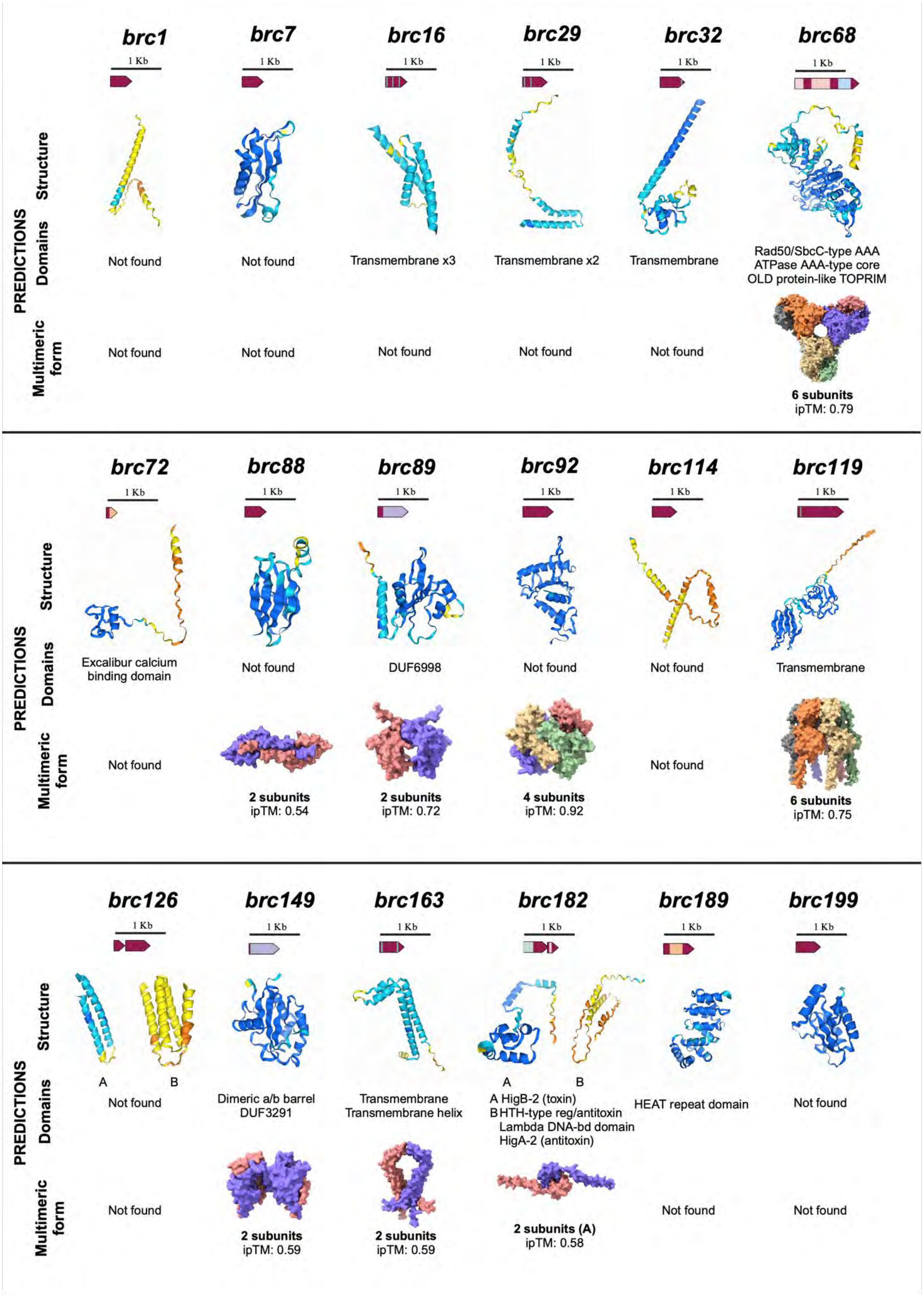


Fig. S2. Heatmap of defense profiles across different phage types. Heatmap showing the defense profiles of identified Bacteriophage Resistance integron Cassettes (BRiCs) against a panel of different phages. Each cell represents the level of inhibition, with 100% indicating total susceptibility (death) and 0% indicating total protection. The colour intensity reflects the degree of inhibition, where dark green represents high protection and blue indicates lower protection. The defense profiles are shown for various BRiCs against multiple phages, demonstrating the specificity and broad spectrum of phage resistance conferred by different *gcus*.



brc1
brc7
brc16
brc29
brc32
brc68
1 Kb
PREDICTIONS
Structure
Domains
Multimeric form
Not found
Not found
Transmembrane x3
Transmembrane x2
Transmembrane
Rad50/SbcC-type AAA
ATPase AAA-type core
OLD protein-like TOPRIM
Not found
Not found
Not found
Not found
Not found
6 subunits
ipTM: 0.79
brc72
brc88
brc89
brc92
brc114
brc119
Excalibur calcium binding domain
Not found
DUF6998
Not found
Not found
Transmembrane
Not found
2 subunits
ipTM: 0.54
2 subunits
ipTM: 0.72
4 subunits
ipTM: 0.92
Not found
6 subunits
ipTM: 0.75
brc126
brc149
brc163
brc182
brc189
brc199
A
B
Not found
Dimeric a/b barrel
DUF3291
Transmembrane
Transmembrane helix
A HigB-2 (toxin)
B HTH-type reg/antitoxin
Lambda DNA-bd domain
HigA-2 (antitoxin)
HEAT repeat domain
Not found
Not found
2 subunits
ipTM: 0.59
2 subunits
ipTM: 0.59
2 subunits (A)
ipTM: 0.58
Not found
Not found

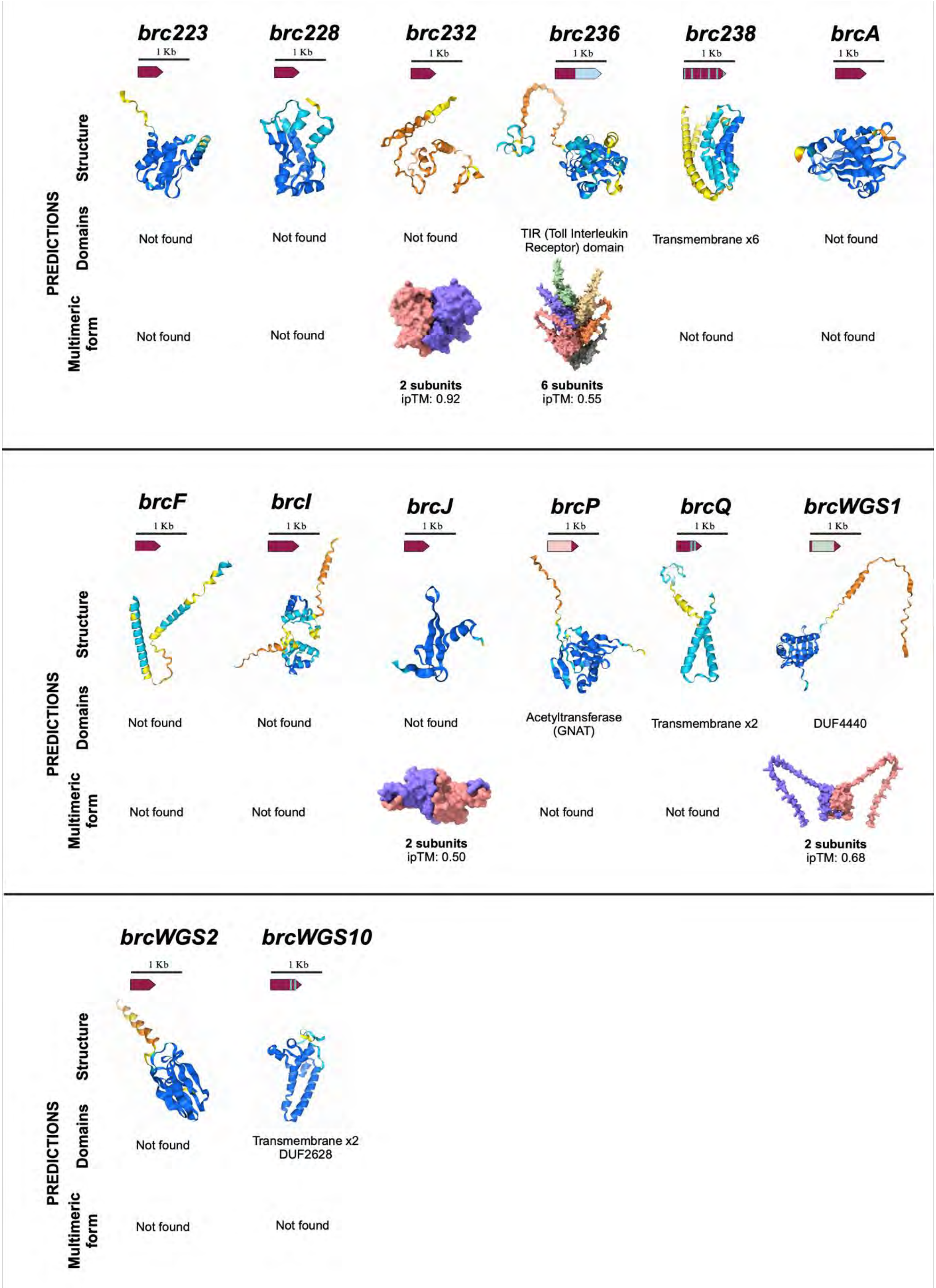


Fig. S3. *gcus* with bacteriophage defense activity: *brcs*. Genetic organization, and predicted function and domains of selected *gcus*. Tertiary and quaternary structures were predicted using AlphaFold3 and visualized with ChimeraX.

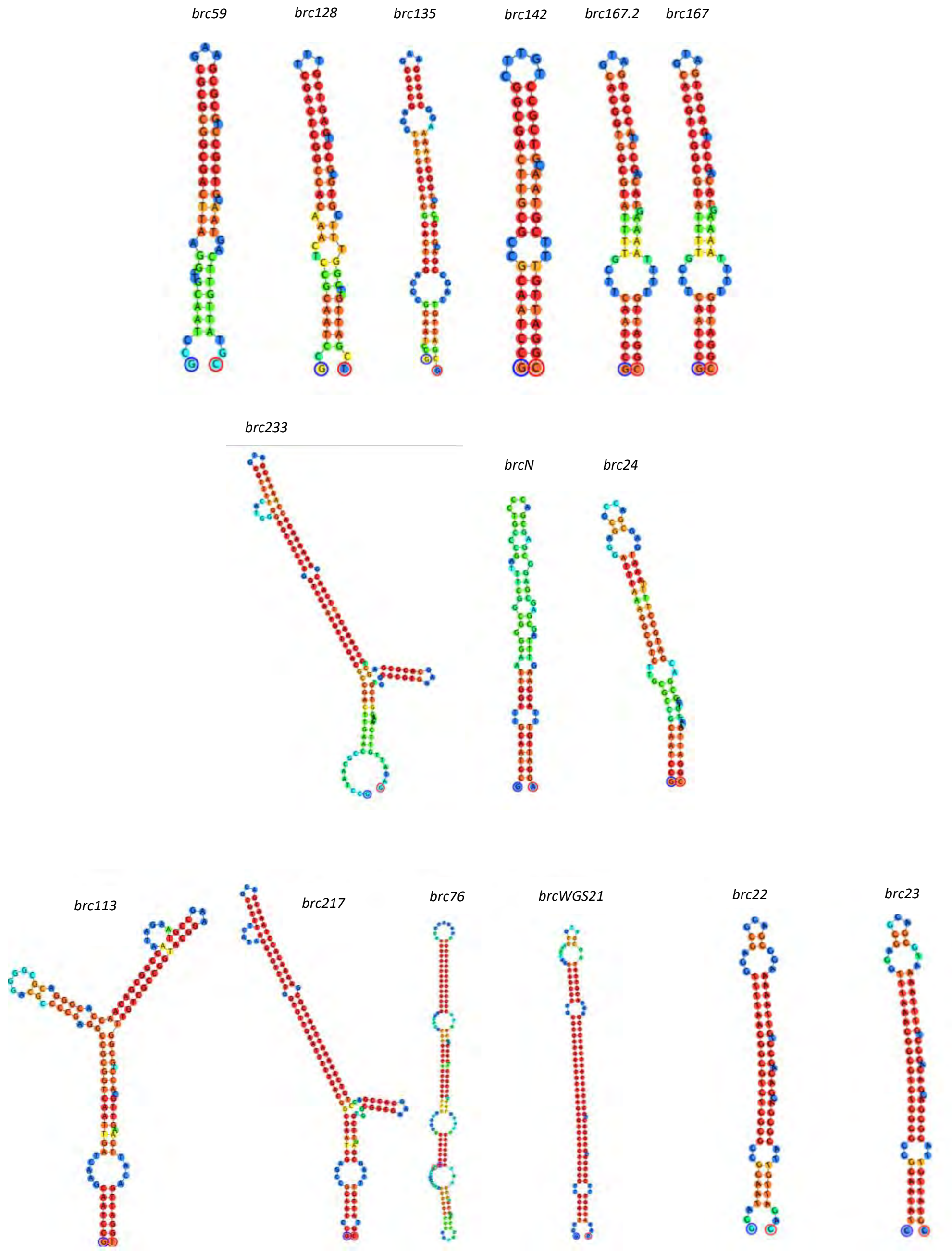
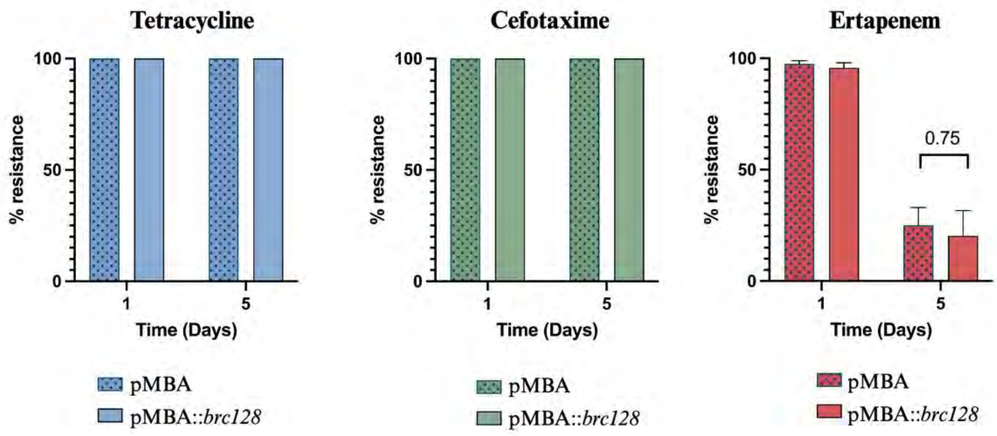


Fig. S4. Predicted secondary structures of *attC* sites in identified BRiCs. Secondary structures of attC sites from various BRiCs are shown. Each structure represents the predicted folding pattern for the *attC* sites associated with each *brc*. The structures are color-coded based on nucleotide positions, with blue representing the outer loops and red indicating the stems of the hairpins. These structural predictions highlight the diversity and complexity of *attC* site configurations, which are critical for site-specific recombination events mediated by integrases.

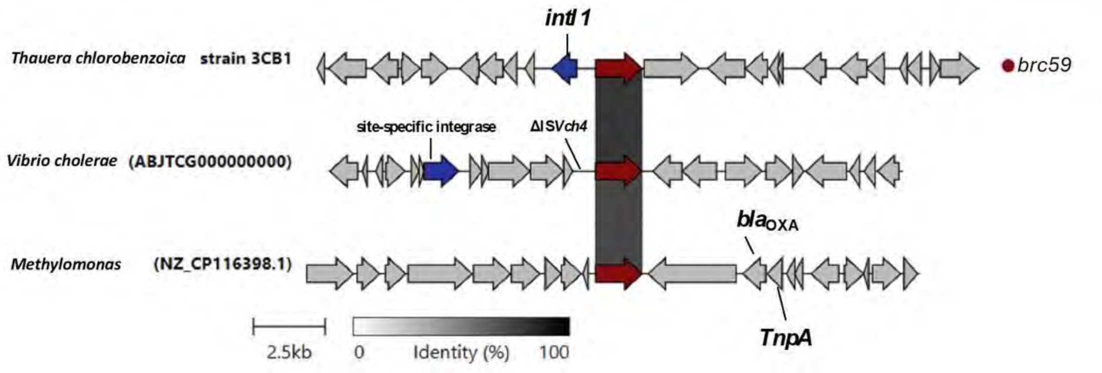


Plasmid content of KP5 *K. pneumoniae* isolate.

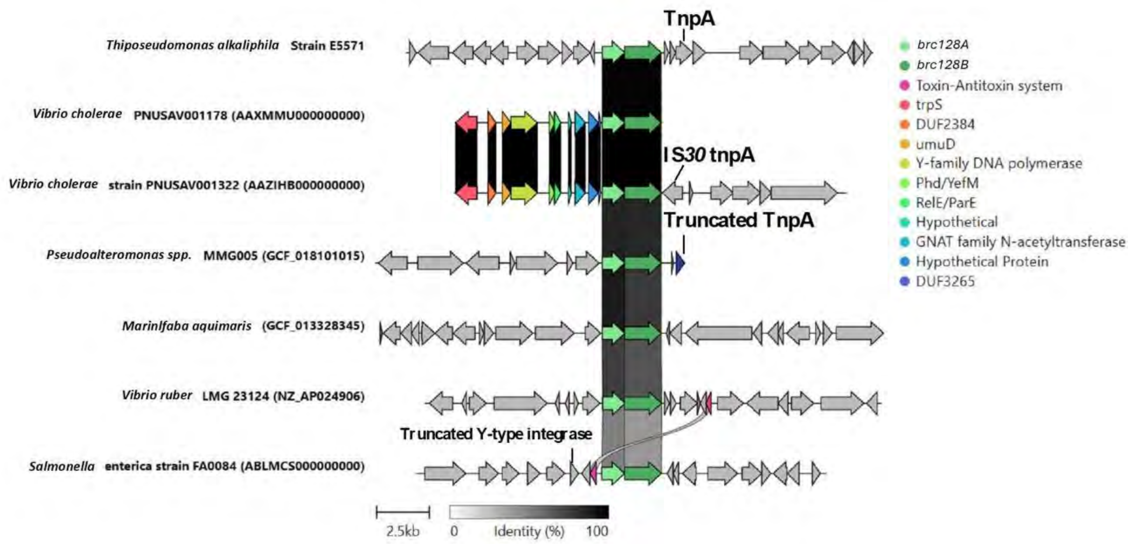
Name	Plasmid type	Resistome
Kp5	IncR (38kb)	*bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *bla*$_{CTX-M-15}$, *sul2*
	IncL (63kb)	*bla*$_{OXA-48}$
	IncH1B(200kb)	*sul1*, *dfrA15*, *aadA1*, *tet(A)* and *catA1*

Fig. S5. Stability of the three natural plasmids found in KP5. Stability of the plasmids encoded in KP5 was followed over ca. 30 generations (5 days) in the presence and absence of *brc128.* Three replicates of each strain were passaged in the absence of antibiotic and plated at every time point in LB without antibiotics. Plasmid stability was measured replicating 40 colonies of each with and without the corresponding antibiotic. Plasmids conferring tetracycline and cefotaxime resistance were 100% stable in all cases. Plasmid conferring ertapenem resistance was not stable, but the differences between the empty pMBA and pMBA harbouring *brc128* were not statistically significant. Bars represent the standard error of the mean.

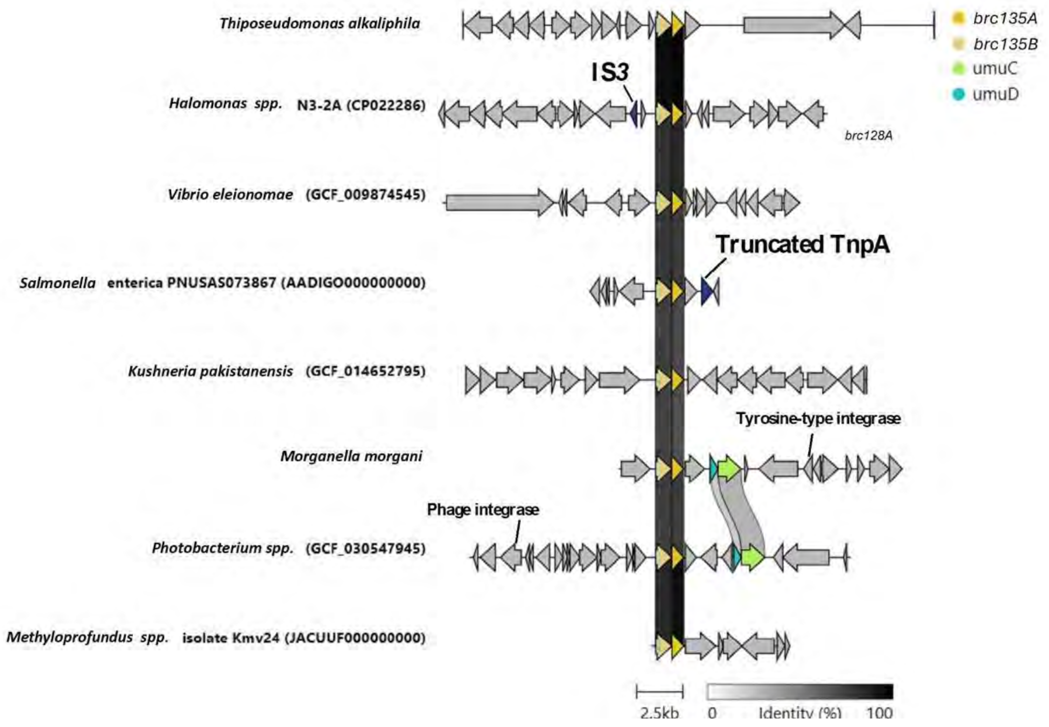
PARIS II



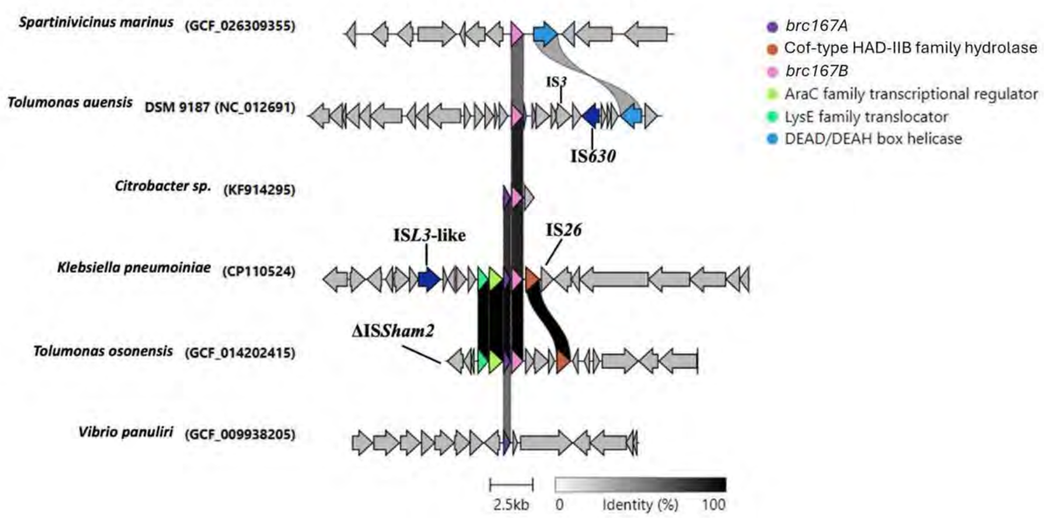
Lamassu I



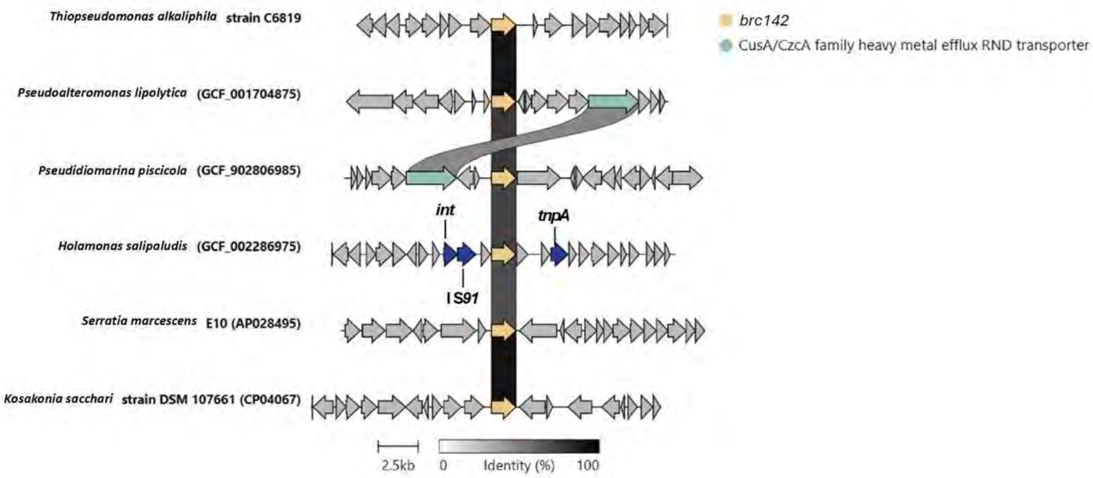
CBASS I



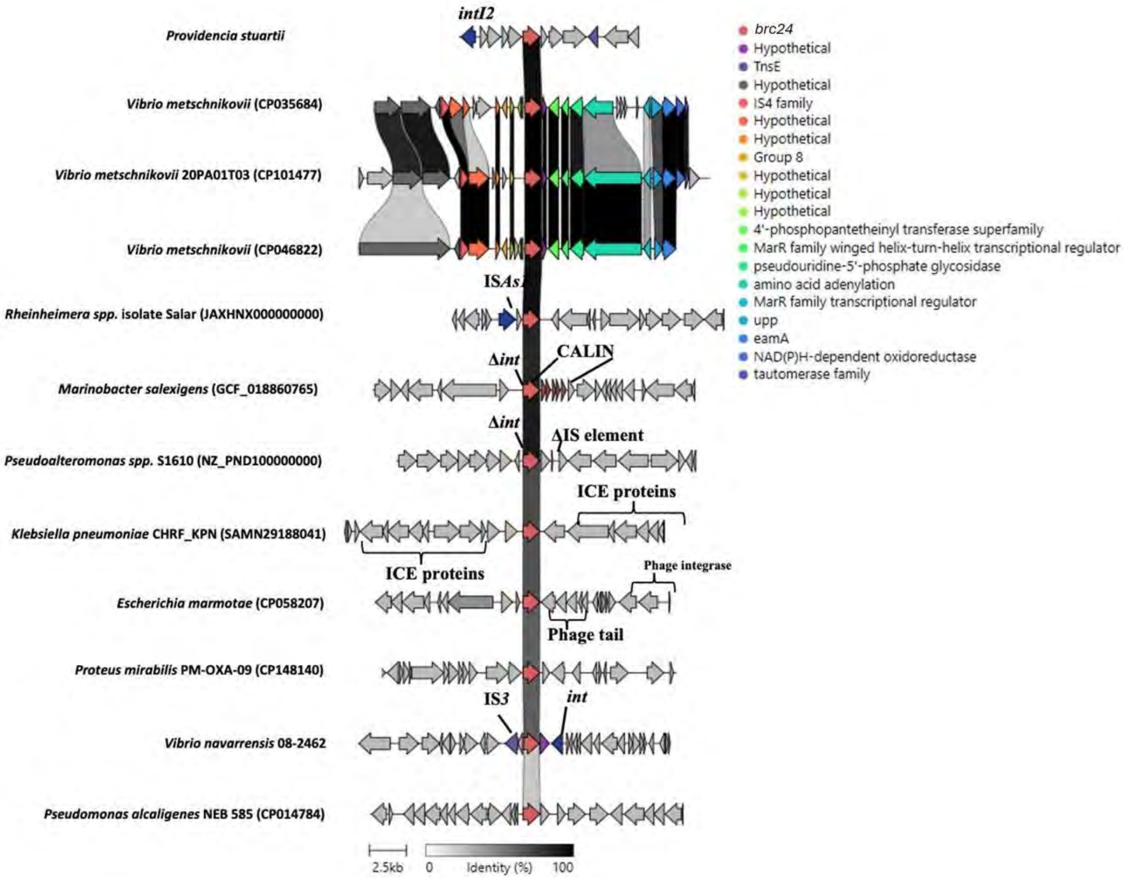
Ataecina



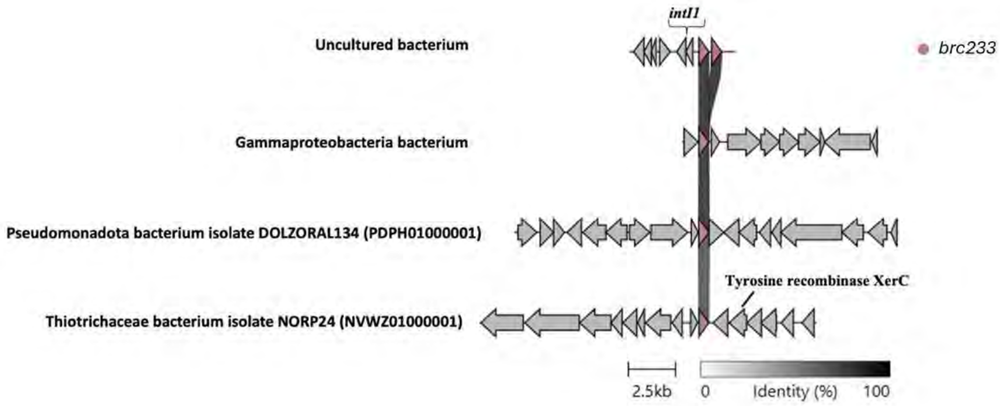
Endovelico



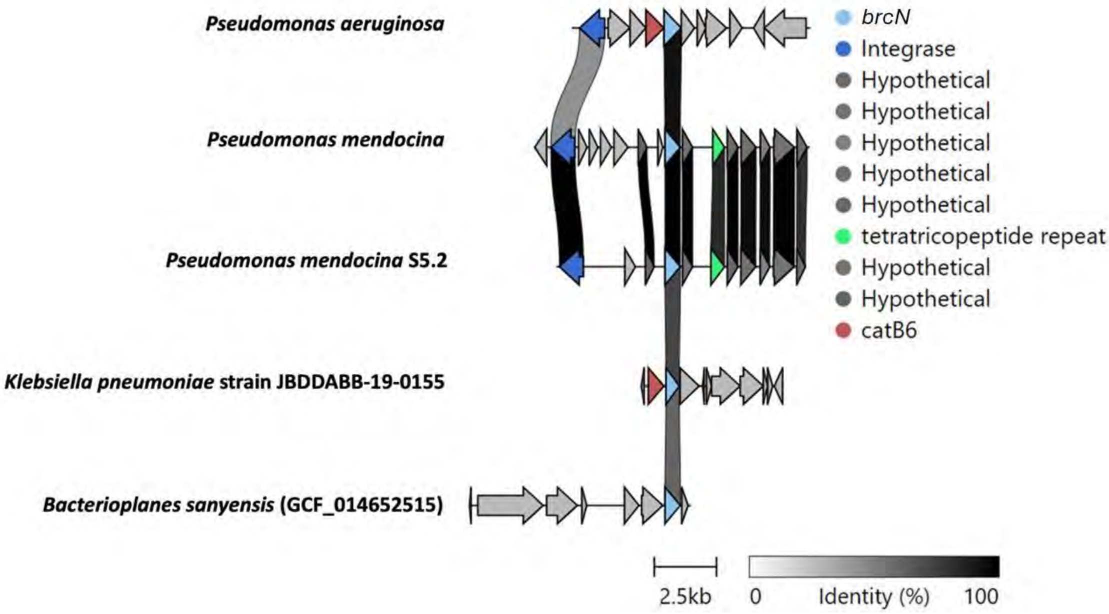
Cosus



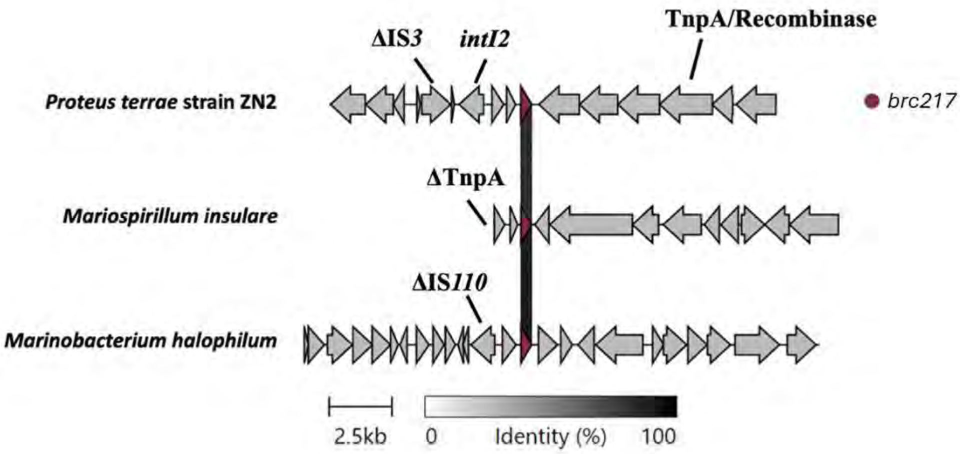
Candamuis



AbiV



Arconi



Tragantía

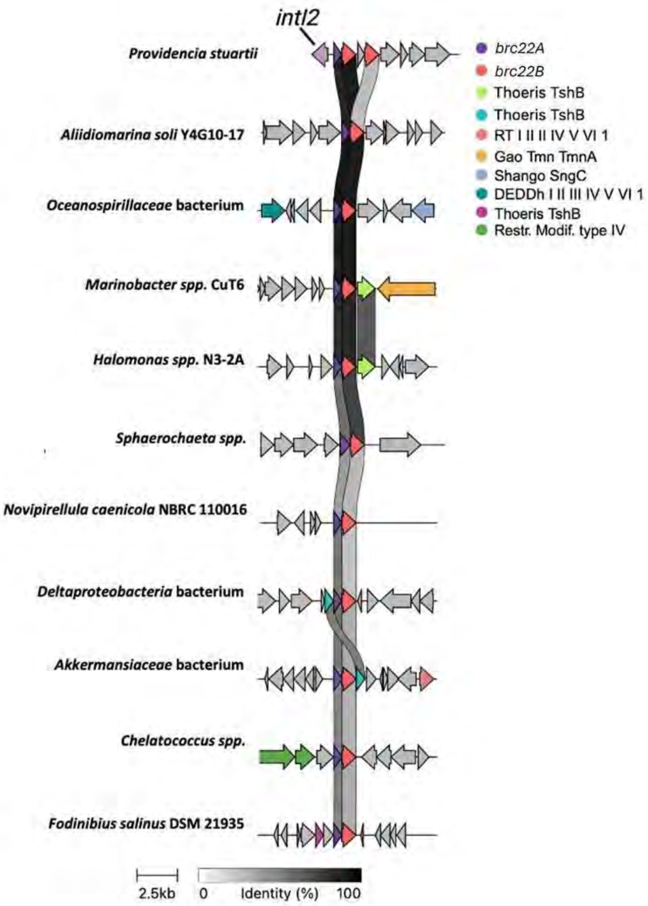
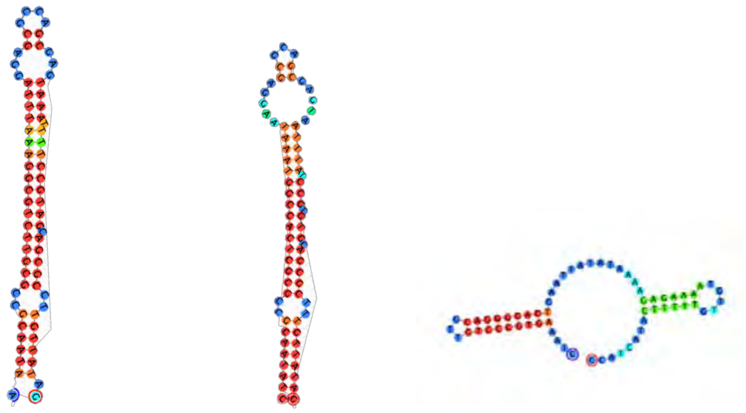


Fig. S6. Genetic contexts of *brc*s. Images show the genetic context of *brc* hits in the databases using CAGECAT (Comparative Gene Cluster Analysis Toolbox).



Providencia stuartii *Pseudoalteromonas spp.* *Escherichia marmotae*

Fig. S7. Predicted secondary structures of *attC* sites identified in Cosus homologs. Each structure represents the predicted folding pattern for the *attC* sites associated with each Cosus homolog. The structures are color-coded based on nucleotide positions, with blue representing the outer loops and red indicating the stems of the hairpins.

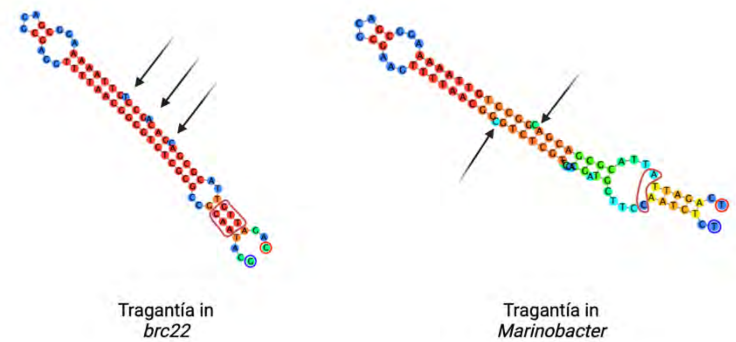


Fig. S8. Predicted secondary structures of *attC* sites identified in Tragantía homologs. Arrows show the extrahelical bases that are recognised by the integrase as landmarks in *attC* sites. The red shapes mark the correct formation of the R box in the *attC* of *brc22*, with a perfect GTT/AAC binding between both arms of the stem. Instead, in the pseudo *attC* in *Marinobacter* there is no GTT/AAC box due to a single mutation, and one extrahelical base is found in the opposite side of the stem.

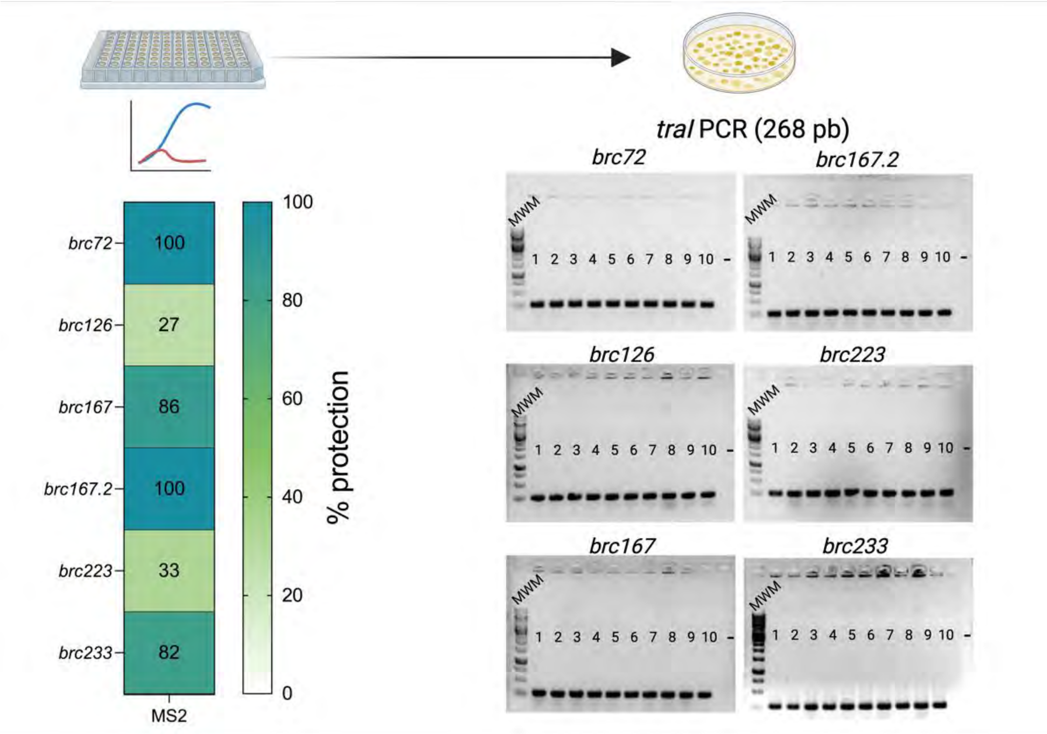


Fig. S9. F'128 is present after challenge with MS2. We measured the growth of 6 BRiCs while challenged with MS2 infection in the presence of tetracycline to force the presence of F'128. We measured resistance and found that it was not abolished in any case, suggesting that it is not related to plasmid loss. We then plated the cultures and performed PCR of the *traI* gene in F'128 to 10 colonies from each assay. All colonies contained F'128.

Table S1. Strains and plasmids used in this work.

Bacterial Strains				
Number	**Plasmids**	**Bacterial species**	**Experiments**	**Reference**
A249	pMBA	*E. coli* MG1655	General use	*Hipólito et al.* 2022
B800	None	*E. coli* IJ1862		*Bull et al.* 2004
B931	IncL, IncR, IncH1A	*K. pneumoniae* KP5		This study
A760	None	*P. aeruginosa* PAO1		Lab collection
A115	None	*E. coli* β2163		*Demarre et al.* 2005
A123	pSW23T::*attC*$_{aadA7}$ bs	*E. coli* β2163		*Demarre et al.* 2005
A785	pSU38Δ::*attI*/p3938	*E. coli* TOP10		Lab collection
C246	None	*E. coli* 594 (prophage φ80)		*Alqurainy et al.* 2023
C247	None	*E. coli* 594 (prophage HK544)		*Alqurainy et al.* 2023
C665	pMBA *gcu175*	*E. coli* DH5α	Screening	This study
C666	pMBA *gcu93*	*E. coli* DH5α		"
C667	pMBA *gcu149*	*E. coli* DH5α		"
C668	pMBA *gcu26c*	*E. coli* DH5α		"
C669	pMBA *gcu173*	*E. coli* DH5α		"
C670	pMBA *gcu80*	*E. coli* DH5α		"
C671	pMBA *gcu153*	*E. coli* DH5α		"
C672	pMBA *gcu179*	*E. coli* DH5α		"
C673	pMBA *gcu168*	*E. coli* DH5α		"
C674	pMBA *gcu79*	*E. coli* DH5α		"
C675	pMBA *gcu18*	*E. coli* DH5α		"
C676	pMBA *gcu145*	*E. coli* DH5α		"
C677	pMBA *gcuWGS7*	*E. coli* DH5α		"
C678	pMBA *gcu32*	*E. coli* DH5α		"
C679	pMBA *gcu88*	*E. coli* DH5α		"
C680	pMBA *gcuA*	*E. coli* DH5α		"
C681	pMBA *gcu228*	*E. coli* DH5α		"
C682	pMBA *gcu170*	*E. coli* DH5α		"
C683	pMBA *gcu20*	*E. coli* DH5α		"
C684	pMBA *gcu87*	*E. coli* DH5α		"
C685	pMBA *gcu17*	*E. coli* DH5α		"
C686	pMBA *gcuWGS5*	*E. coli* DH5α		"
C687	pMBA *gcu211*	*E. coli* DH5α		"
C688	pMBA *gcu144*	*E. coli* DH5α		"
C689	pMBA *gcu28*	*E. coli* DH5α		"
C690	pMBA *gcuI*	*E. coli* DH5α	Screening	"

C691	pMBA *gcuC*	*E. coli* DH5α		"
C692	pMBA *gcu19*	*E. coli* DH5α		"
C693	pMBA *gcu12*	*E. coli* DH5α		"
C694	pMBA *gcu225*	*E. coli* DH5α		"
C695	pMBA *gcuWGS10*	*E. coli* DH5α		"
C696	pMBA *gcuWGS15*	*E. coli* DH5α		"
C697	pMBA *gcu72*	*E. coli* DH5α		"
C698	pMBA *gcu189*	*E. coli* DH5α		"
C699	pMBA *gcu100*	*E. coli* DH5α		"
C700	pMBA *gcuJ*	*E. coli* DH5α		"
C701	pMBA *gcu1*	*E. coli* DH5α		"
C702	pMBA *gcu107*	*E. coli* DH5α		"
C703	pMBA *gcu76c*	*E. coli* DH5α		"
C704	pMBA *gcuWGS2*	*E. coli* DH5α		"
C705	pMBA *gcu76*	*E. coli* DH5α		"
C706	pMBA *gcuP*	*E. coli* DH5α		"
C707	pMBA *gcu78*	*E. coli* DH5α		"
C708	pMBA *gcu7*	*E. coli* DH5α		"
C709	pMBA *gcu57*	*E. coli* DH5α		"
C710	pMBA *gcu232*	*E. coli* DH5α		"
C711	pMBA *gcu221*	*E. coli* DH5α		"
D153	pMBA *gcu23*	*E. coli* DH5α		"
C713	pMBA *gcu114*	*E. coli* DH5α		"
C714	pMBA *gcuQ*	*E. coli* DH5α		"
C715	pMBA *gcu11*	*E. coli* DH5α		"
C716	pMBA *gcu89*	*E. coli* DH5α		"
C717	pMBA *gcu92*	*E. coli* DH5α		"
C718	pMBA *gcu96*	*E. coli* DH5α		"
C720	pMBA *gcuF*	*E. coli* DH5α		"
C721	pMBA *gcu199*	*E. coli* DH5α		"
C722	pMBA *gcu65*	*E. coli* DH5α		"
C723	pMBA *gcu217*	*E. coli* DH5α		"
C724	pMBA *gcu238*	*E. coli* DH5α		"
C725	pMBA *gcu236*	*E. coli* DH5α		"
C726	pMBA *gcuWGS1*	*E. coli* DH5α		"
D148	pMBA *gcu14*	*E. coli* DH5α		"
C728	pMBA *gcu177*	*E. coli* DH5α		"
C729	pMBA *gcu227*	*E. coli* DH5α		"
C730	pMBA *gcuWGS21*	*E. coli* DH5α		"
C731	pMBA *gcu185*	*E. coli* DH5α		"
C732	pMBA *gcu231*	*E. coli* DH5α		"
C733	pMBA *gcu46*	*E. coli* DH5α		"
C734	pMBA *gcu9*	*E. coli* DH5α		"
C735	pMBA *gcu83*	*E. coli* DH5α		"
C736	pMBA *gcu48*	*E. coli* DH5α	Screening	"

C737	pMBA *gcu113*	*E. coli* DH5α		"
C738	pMBA *gcu197*	*E. coli* DH5α		"
C739	pMBA *gcuWGS3*	*E. coli* DH5α		"
C740	pMBA *gcu262*	*E. coli* DH5α		"
C741	pMBA *gcu184*	*E. coli* DH5α		"
C742	pMBA *gcu137*	*E. coli* DH5α		"
C743	pMBA *gcu143*	*E. coli* DH5α		"
C744	pMBA *gcu141*	*E. coli* DH5α		"
C745	pMBA *gcu142*	*E. coli* DH5α		"
C746	pMBA *gcu121*	*E. coli* DH5α		"
C747	pMBA *gcu24*	*E. coli* DH5α		"
C748	pMBA *gcu8*	*E. coli* DH5α		"
C749	pMBA *gcu109*	*E. coli* DH5α		"
C750	pMBA *gcu101*	*E. coli* DH5α		"
C751	pMBA *gcu21*	*E. coli* DH5α		"
C752	pMBA *gcu77*	*E. coli* DH5α		"
C753	pMBA *gcu230*	*E. coli* DH5α		"
C754	pMBA *gcuH*	*E. coli* DH5α		"
C755	pMBA *gcu163*	*E. coli* DH5α		"
C756	pMBA *gcu223*	*E. coli* DH5α		"
C757	pMBA *gcu29*	*E. coli* DH5α		"
C758	pMBA *gcu190*	*E. coli* DH5α		"
C759	pMBA *gcu172*	*E. coli* DH5α		"
C760	pMBA *gcu16*	*E. coli* DH5α		"
C761	pMBA *gcu108*	*E. coli* DH5α		"
C762	pMBA *gcu196*	*E. coli* DH5α		"
C763	pMBA *gcu5b*	*E. coli* DH5α		"
C764	pMBA *gcu37*	*E. coli* DH5α		"
C765	pMBA *gcu135*	*E. coli* DH5α		"
C766	pMBA *gcuWGS6*	*E. coli* DH5α		"
C767	pMBA *gcu253A*	*E. coli* DH5α		"
C768	pMBA *gcu133*	*E. coli* DH5α		"
C769	pMBA *gcu158*	*E. coli* DH5α		"
C770	pMBA *gcu39*	*E. coli* DH5α		"
C771	pMBA *gcu74*	*E. coli* DH5α		"
C149	pMBA *gcu157*	*E. coli* DH5α		"
C773	pMBA *gcu122*	*E. coli* DH5α		"
C774	pMBA *gcu128*	*E. coli* DH5α		"
C775	pMBA *gcu59*	*E. coli* DH5α		"
C776	pMBA *gcu154A*	*E. coli* DH5α		"
D150	pMBA *gcu138*	*E. coli* DH5α		"
C778	pMBA *gcu118*	*E. coli* DH5α		"
C779	pMBA *gcu68*	*E. coli* DH5α		"
C780	pMBA *gcu52*	*E. coli* DH5α		"
C782	pMBA *gcuWGS13*	*E. coli* DH5α	Screening	"

C784	pMBA *gcu182B*	*E. coli* DH5α		“
C785	pMBA *gcu233*	*E. coli* DH5α		“
C786	pMBA *gcu22A*	*E. coli* DH5α		“
C788	pMBA *gcu41*	*E. coli* DH5α		“
C789	pMBA *gcu43*	*E. coli* DH5α		“
C790	pMBA *gcu26b*	*E. coli* DH5α		“
C792	pMBA *gcu6*	*E. coli* DH5α		“
C793	pMBA *gcu111*	*E. coli* DH5α		“
C795	pMBA *gcu102*	*E. coli* DH5α		“
C796	pMBA *gcu54*	*E. coli* DH5α		“
C797	pMBA *gcu117*	*E. coli* DH5α		“
C798	pMBA *gcu119*	*E. coli* DH5α		“
C799	pMBA *gcu136*	*E. coli* DH5α		“
D383	pMBA-*brcI*	*E. coli* IJ1862		“
D398	pMBA-*brcWGS10*	*E. coli* IJ1862		“
D400	pMBA-*brc72*	*E. coli* IJ1862		“
D403	pMBA-*brc1*	*E. coli* IJ1862		“
D410	pMBA-*brc7*	*E. coli* IJ1862		“
D478	pMBA-*brc16*	*E. coli* IJ1862		“
D475	pMBA-*brc29*	*E. coli* IJ1862		“
D414	pMBA-*brc32*	*E. coli* IJ1862		“
D493	pMBA-*brc68*	*E. coli* IJ1862		“
D441	pMBA-*brc88*	*E. coli* IJ1862		“
D418	pMBA-*brc89*	*E. coli* IJ1862		“
D419	pMBA-*brc92*	*E. coli* IJ1862		“
D415	pMBA-*brc114*	*E. coli* IJ1862		“
D508	pMBA-*brc119*	*E. coli* IJ1862		“
D517	pMBA-*brc126*	*E. coli* IJ1862		“
D431	pMBA-*brc149*	*E. coli* IJ1862	Positive BRiCs in the screening against the complete panel of phages	“
D473	pMBA-*brc163*	*E. coli* IJ1862		“
D497	pMBA-*brc182*	*E. coli* IJ1862		“
D401	pMBA-*brc189*	*E. coli* IJ1862		“
D422	pMBA-*brc199*	*E. coli* IJ1862		“
D474	pMBA-*brc223*	*E. coli* IJ1862		“
D443	pMBA-*brc228*	*E. coli* IJ1862		“
D412	pMBA-*brc232*	*E. coli* IJ1862		“
D425	pMBA-*brc236*	*E. coli* IJ1862		“
D424	pMBA-*brc238*	*E. coli* IJ1862		“
D442	pMBA-*brcA*	*E. coli* IJ1862		“
D421	pMBA-*brcF*	*E. coli* IJ1862		“
D402	pMBA-*brcJ*	*E. coli* IJ1862		“
D408	pMBA-*brcP*	*E. coli* IJ1862		“
D416	pMBA-*brcQ*	*E. coli* IJ1862		“
D426	pMBA-*brcWGS1*	*E. coli* IJ1862		“
D406	pMBA-*brcWGS2*	*E. coli* IJ1862		“

D529	pMBA-*brc24*-T12A	*E. coli* DH5α	Positive BRiCs in the screening against the complete panel of phages	“
D534	pMBA-*brc24*-D96A	*E. coli* DH5α		“
D538	pMBA-*brc24*-D14A	*E. coli* DH5α		“
C809	pMBA	*E. coli* IJ1862	High/low MOI Phage panel	“
C800	pMBA *brc59*	*E. coli* IJ1862		“
C801	pMBA *brc128*	*E. coli* IJ1862		“
C802	pMBA *brc135*	*E. coli* IJ1862		“
C803	pMBA *brc167*	*E. coli* IJ1862		“
C804	pMBA *brc167.2*	*E. coli* IJ1862		“
C805	pMBA *brc142*	*E. coli* IJ1862		“
C806	pMBA *brc24*	*E. coli* IJ1862		“
C807	pMBA *brc233*	*E. coli* IJ1862		“
C808	pMBA *brcN*	*E. coli* IJ1862		“
C933	pMBA *brc76*	*E. coli* IJ1862		“
C934	pMBA *brc217*	*E. coli* IJ1862		“
C935	pMBA *brcWGS21*	*E. coli* IJ1862		“
C936	pMBA *brc113*	*E. coli* IJ1862		“
C819	pMBA	*K. pneumoniae* KP5	BRiCs in other species	“
C810	pMBA *brc59*	*K. pneumoniae* KP5		“
C811	pMBA *brc128*	*K. pneumoniae* KP5		“
C812	pMBA *brc135*	*K. pneumoniae* KP5		“
C813	pMBA *brc167*	*K. pneumoniae* KP5		“
C814	pMBA *brc167.2*	*K. pneumoniae* KP5		“
C815	pMBA *brc142*	*K. pneumoniae* KP5		“
C816	pMBA *brc24*	*K. pneumoniae* KP5		“
C817	pMBA *brc233*	*K. pneumoniae* KP5		“
C818	pMBA *brcN*	*K. pneumoniae* KP5		“
C939	pMBA *brc76*	*K. pneumoniae* KP5		“
C940	pMBA *brc217*	*K. pneumoniae* KP5		“
C941	pMBA *brcWGS21*	*K. pneumoniae* KP5		“
C942	pMBA *brc113*	*K. pneumoniae* KP5		“
C971	pBTZ PcW *brc24*	*P. aeruginosa* PAO1		“
C972	pBTZ PcW *brc142*	*P. aeruginosa* PAO1		“

C973	pBTZ PcW *brcWGS21*	*P. aeruginosa* PAO1	BRiCs in other species	"
C974	pBTZ PcW *brc113*	*P. aeruginosa* PAO1		"
C975	pBTZ-PcW	*P. aeruginosa* PAO1		"
C898	pBTZ PcS *brcN*	*P. aeruginosa* PAO1		"
C915	pMBA $\textit{bla}_{\text{OXA-10}}$	*E. coli* IJ1862	Multicassette arrays	"
C916	pMBA $\textit{brc24-bla}_{\text{OXA-10}}$	*E. coli* IJ1862		"
D159	pMBA *brc24-brc167.2*	*E. coli* IJ1862		"
D379	pMBA *aacA54-aacA8-brc24*	*E. coli* DH5α		"
D022	pMBA-ΔGFP	*E. coli* IJ1862	Fitness cost measurement	"
C962	pMBA-ΔGFP *brc76*	*E. coli* IJ1862		"
D063	pMBA-ΔGFP *brc113*	*E. coli* IJ1862		"
D068	pMBA-ΔGFP *brc217*	*E. coli* IJ1862		"
D023	pMBA-ΔGFP *brc76*	*E. coli* IJ1862		"
D024	pMBA-ΔGFP *brc128*	*E. coli* IJ1862		"
D025	pMBA-ΔGFP *brc135*	*E. coli* IJ1862		"
D026	pMBA-ΔGFP *brc167*	*E. coli* IJ1862		"
D027	pMBA-ΔGFP *brc142*	*E. coli* IJ1862		"
D028	pMBA-ΔGFP *brc24*	*E. coli* IJ1862		"
D029	pMBA-ΔGFP *brcN*	*E. coli* IJ1862		"
D064	pMBA-ΔGFP *brc167.2*	*E. coli* IJ1862		"
D065	pMBA-ΔGFP *brcWGS21*	*E. coli* IJ1862		"
D066	pMBA-ΔGFP *brc59*	*E. coli* IJ1862		"
D067	pMBA-ΔGFP *brc233*	*E. coli* IJ1862		"
D068	pMBA-ΔGFP *brc217*	*E. coli* IJ1862		"
D063	pMBA-ΔGFP *brc113*	*E. coli* IJ1862		"
D092	pMBA-ΔGFP *brcN*	*K. pneumoniae* KP5		"
D093	pMBA-ΔGFP *brc59*	*K. pneumoniae* KP5		"
D094	pMBA-ΔGFP *brc128*	*K. pneumoniae* KP5		"
D095	pMBA-ΔGFP *brcWGS21*	*K. pneumoniae* KP5		"
D096	pMBA-ΔGFP *brc24*	*K. pneumoniae* KP5		"
D097	pMBA-ΔGFP *brc76*	*K. pneumoniae* KP5		"
D098	pMBA-ΔGFP *brc142*	*K. pneumoniae* KP5		"
D099	pMBA-ΔGFP *brc113*	*K. pneumoniae* KP5		"
D100	pMBA-ΔGFP	*K. pneumoniae* KP5		"
D101	pMBA-ΔGFP *brc167.2*	*K. pneumoniae* KP5		"

D102	pMBA-ΔGFP *brc167*	*K. pneumoniae* KP5	Fitness cost measurement	“
D103	pMBA-ΔGFP *brc217*	*K. pneumoniae* KP5		“
D104	pMBA-ΔGFP *brc135*	*K. pneumoniae* KP5		“
D105	pMBA-ΔGFP *brc233*	*K. pneumoniae* KP5		“
D002	pMBA	*E. coli* 594 (prophage φ80)	Prophage induction	“
D286	pMBA *brc59*	*E. coli* 594 (prophage φ80)		“
D016	pMBA *brc128*	*E. coli* 594 (prophage φ80)		“
C998	pMBA *brc135*	*E. coli* 594 (prophage φ80)		“
D018	pMBA *brc167*	*E. coli* 594 (prophage φ80)		“
D011	pMBA *brc167.2*	*E. coli* 594 (prophage φ80)		“
D001	pMBA *brc142*	*E. coli* 594 (prophage φ80)		“
D010	pMBA *brc24*	*E. coli* 594 (prophage φ80)		“
C999	pMBA *brc233*	*E. coli* 594 (prophage φ80)		“
D021	pMBA *brcN*	*E. coli* 594 (prophage φ80)		“
C957	pMBA *brc76*	*E. coli* 594 (prophage φ80)		“
C951	pMBA *brc217*	*E. coli* 594 (prophage φ80)		“
C948	pMBA *brcWGS21*	*E. coli* 594 (prophage φ80)		“
C954	pMBA *brc113*	*E. coli* 594 (prophage φ80)		“
C996	pMBA	*E. coli* 594 (prophage HK544)		“
D285	pMBA *brc59*	*E. coli* 594 (prophage HK544)		“
D008	pMBA *brc128*	*E. coli* 594 (prophage HK544)		“
C995	pMBA *brc135*	*E. coli* 594 (prophage HK544)		“
D017	pMBA *brc167*	*E. coli* 594 (prophage HK544)		“
D019	pMBA *brc167.2*	*E. coli* 594 (prophage HK544)		“
C997	pMBA *brc142*	*E. coli* 594 (prophage HK544)		“
D003	pMBA *brc24*	*E. coli* 594 (prophage HK544)		“
C994	pMBA *brc233*	*E. coli* 594 (prophage HK544)		“

D020	pMBA *brcN*	*E. coli* 594 (prophage HK544)	Prophage induction	"
C959	pMBA *brc76*	*E. coli* 594 (prophage HK544)		"
C953	pMBA *brc217*	*E. coli* 594 (prophage HK544)		"
C950	pMBA *brcWGS21*	*E. coli* 594 (prophage HK544)		"
C956	pMBA *brc113*	*E. coli* 594 (prophage HK544)		"
B918	pSW23T:: *brc59* bs	*E. coli* β2163	Cassette recombination assays	"
B920	pSW23T::*brc128* bs	*E. coli* β2163		"
B924	pSW23T::*brc135* bs	*E. coli* β2163		"
D291	pSW23T::*brcN* bs	*E. coli* β2163		"
C984	pSW23T::*brc24* bs	*E. coli* β2163		"
C988	pSW23T::*brc76* bs	*E. coli* β2163		"
C986	pSW23T::*brc113* bs	*E. coli* β2163		"
D292	pSW23T::*brc142* bs	*E. coli* β2163		"
B922	pSW23T::*brc167* bs	*E. coli* β2163		"
D293	pSW23T::*brc167.2* bs	*E. coli* β2163		"
C985	pSW23T::*brc217* bs	*E. coli* β2163		"
D294	pSW23T::*brc233* bs	*E. coli* β2163		"
C987	pSW23T::*brcWGS21* bs	*E. coli* β2163		"
B917	pSW23T:: *brc59* ts	*E. coli* β2163		"
B919	pSW23T::*brc128* ts	*E. coli* β2163		"
B923	pSW23T::*brc135* ts	*E. coli* β2163		"
D295	pSW23T::*brcN* ts	*E. coli* β2163		"
C989	pSW23T::*brc24* ts	*E. coli* β2163		"
C993	pSW23T::*brc76* ts	*E. coli* β2163		"
C991	pSW23T::*brc113* ts	*E. coli* β2163		"
D296	pSW23T::*brc142* ts	*E. coli* β2163		"
B921	pSW23T::*brc167* ts	*E. coli* β2163		"
D297	pSW23T::*brc167.2* ts	*E. coli* β2163		"
C990	pSW23T::*brc217* ts	*E. coli* β2163		"
D298	pSW23T::*brc233* ts	*E. coli* β2163		"
C992	pSW23T::*brcWGS21* ts	*E. coli* β2163		"
D278	pMBA PcW *brc22*	*E. coli* IJ1862	Naturally occurring defense islands	"
D279	pMBA PcW *brc23*	*E. coli* IJ1862		"
D283	pMBA PcW *brc22*	*K. pneumoniae* KP5		"
D284	pMBA PcW *brc23*	*K. pneumoniae* KP5		"
D287	pMBA PcW *brc23-brc24*	*E. coli* DH5α		"
D289	pMBA PcW *brc23-brc24*	*E. coli* IJ1862		"

Table S2. Oligonucleotides Used for Cloning and Amplification.

Name	Sequence 5'-> 3'	Role
gBLOCK F	GCAGTCGCCCTAAAACAAAG	Amplifying *gcu*s for cloning in pMBA
gBLOCK R	GCTGCAGCGTCGACGCCTAA	
GFP F BackBone pMBA	TTAGGCGTCGACGCTGCA	Amplify pMBA backbone
Int R BackBone pMBA	CTTTGTTTTAGGGCGACTGC	
Array-Ø F	GCAGTCGCCCTAAAACAAAGTTA GGCCGCACAAAATCAACG	Amplify *aacA54* and *aacA8* to clone it into pMBA KO
Array-Ø R	GCTGCAGCGTCGACGCCTAACGT TTGACATGAGGGGCGGC	
brc24-ArrayØ F	GCCGCCCCTCATGTCAAACGTTA TAGCTCAAGCAGCTCATCTCG	Amplify *brc24* to clone it in pArray Ø
brc24-ArrayØ R	GCTGCAGCGTCGACGCCTAACGC CGCGTTCTGCGGAAATT	
BackBone pArray *brc24* R	CGTTTGACATGAGGGGCGGC	Used with GFP_F_BackBone_pMBA on pArrayØ to amplify the backbone to clone *brc24*
BB pSW23T GA-R	ACTTTGTTTTAGGGCGACTGCGAT ATCAAGCTTATCGATAC	Amplify pSW23T backbone to clone *gcu*s amplified with gBLOCK F and gBLOCK R, to deliver the bottom strand
BB pSW23T GA-F	TTAGGCGTCGACGCTGCAGCACT AGTTCTAGAGCGGCCGC	
BB pSW23T AG-R	TTAGGCGTCGACGCTGCAGCGAT ATCAAGCTTATCGATAC	Amplify pSW23T backbone to clone *gcu*s amplified with gBLOCK F and gBLOCK R, to deliver the top strand
BB pSW23T AG-F	ACTTTGTTTTAGGGCGACTGCACT AGTTCTAGAGCGGCCGC	
brc128 pBAD tail F	TTAACCATGGATCCGAGCTCATG GTAAATAGTAATTCTGGTGTAGA GG	Clone *brc128AB* into pBAD
brc128 pBAD tail R	TGAGATGAGTTTTTGTTCTAGAG GATATTGAAATATTAAGTTAG	
pBAD BBExt F	TCTAGAACAAAAACTCATCTC	Amplify pBAD backbone
pBAD BBExt R	GAGCTCGGATCCATGGTTAA	
gcu24-BB 167-2	CGCCGCGTTCTGCGGAAATTT	Backbone amplification to create pArray1 (*brc24-brc167.2*). Use with GFP_F_BackBone_pMBA
167-2 for p24 F	AATTTCCGCAGAACGCGGCGTTA GGCTTTAAAACCATCACTTCCG	Amplify *brc167.2* to clone in second position to create pArray-1
167-2 for p24 R	GCTGCAGCGTCGACGCCTAACTT CGTTTTATGCGGTGGCA	
DdmABC pBAD F	TTAACCATGGATCCGAGCTCGAG AATCGAGTTGCTATTGGT	Amplify *ddmABC* for cloning in pBAD
DdmABC pBAD R	TGAGATGAGTTTTTGTTCTAGAGC ACAACTTGTAAGATAGCCTTGC	
gcu24 for 22/23 F	AATTTGCCGCAGAGCGCGGCGTT ATAGCTCAAGCAGCTCATCTC	Amplify *brc24* to clone it in pMBA::*brc23*
gcu22/23 for 23/24 R	TAACGCCGCGCTCTGCG	
D6A-ext-5	AAATAGAATTAGCTTTTGGCGCG ATAACCATAGATAACGC	Used to mutate *brc24*
D6A-ext-3	GCCAAAAGCTAATTCTATTTCGTC CATAAAACCTTCACGTTTC	"
T12A-ext-5	GATTTTGGCGCGATAGCCATAGA TAACGCGACCTATAAGG	"
T12A-ext-3	ATGGCTATCGCGCCAAAATCTAA TTCTATTTCGTCCATAAAAC	"

D14A-ext-5	GCGTTAGCTATGGTTATATCGCG CCAAAATCTAATTCTATTTCG	"
D14A-ext-3	GCGATAACCATAGCTAACGCGAC CTATAAGGGCGAAGGTTATAG	"
E42A-ext-5	GGGCTTGCTTTAAATTGGCGCAT CTGAGCAAGTAAGCCCCTC	"
E42A-ext-3	CGCCAATTTAAAGCAAGCCCAGT TAAAGTTCTTCAAACCG	"
D47A-ext-5	GTGCACTATAGCGGTTTGAAGAA CTTTAACTGGGCTTTCTTTAAATT GG	"
D47A-ext-3	CTTCAAACCGCTATAGTGCACAA TGAAGCGATCAATCATATC	"
S67A-ext-5	CTGCTCAATCGCGGATCGGGTTTT TGATACTCTTGCCCG	"
S67A-ext-3	CCCGATCCGCGATTGAGCAGGCA CTAAGGTCAGAAATAAAC	"
E69A-ext-5	CTGCGCAATCGAGGATCGGGTTT TTGATATCTCTTGCCCG	"
E69A-ext-3	CCCGATCCTCGATTGCGCAGGCA CTAAGGTCAGCAAATAAAC	"
D96A-ext-5	CTTCGCTGCCAGCAACTGATAGC AACGCTCGAGCATTTTC	"
D96A-ext-3	ATCAGTTGCTGGCAGCGAAGCTG AAATAGCGGAAGCGAGG	"
D120A-ext-5	CTTCCAGAATCGAGCTTCTCAGCT CCAATAAATTCATAATATTTTTC	"
D120A-ext-3	GAGAAGCTCGATTCTGGAAGATA TGCAGATCTATCTCGTC	"
D146A-ext-5	TTTTTGGCCTTTCCGGATTCAAAA GGAGCTTCTGTAGCAAAATAC	"
D146A-ext-3	GAATCCGGAAAGGCCAAAAAAA ATGAATTTCCAGATGCGA	"
D153A-ext-5	AATAGTGCAATCGCAGCTGGAAA TTCATTTTTTTGTCCTTTCCGG	"
D153A-ext-3	CCAGCTGCGATTGCACTATTAGC CCTTGAAGGTTGGGCTG	"
D177A-ext-5	CCTTAGCTTGACTTACGGCGATA ATATTAATTTCATTTTCTTCAGCC CAAC	"
D177A-ext-3	CGCCGTAAGTCAAGCTAAGGGCT GGAAGAACTTTTCTGAAG	"
TraI F	GCCATTCATC TTGCCCTTCC	Amplification of the *traI* gene from F'128 from Wan et al. 2011.
TraI R	GCATGACCGCCTCCTTACC	

Table S3. List of the *gcu* sequences with bacteriophage defense activity. In green are the ORFs. When there is an overlap between ORFs the sequence is underlined.

gcu1

TTAGGCTTGTTGGAATACACCAGTATTCATCGGCCTTGTGGGGAGCCTGTCCTGCGGCAGTCGTCCGCACCACCGAGCAGCTCGGGCTCAGTG
CTGGCAGGGCTTCATCGCAGGTTGAAGCTGGGTTAGATTGTGGTCATCGTCAGCGCATGGCTGACGCCATACGCAAAGGGAGGCGTGATGGC
AAGGTTCGGTTGGGCAACCACCACAGCACATCGCACACACCAGGCTGTCGCCTCGTGTGCGGGCTCGCTGTCCGCGCGACCACCGGCTTACTT
CCACCAGCCTAACTGGTCGGTCAAGCGGACGCCAACACAGGCCATGCCTTCGGCATTCTCATGGCCTGTGTCGGTGCCCTACGCCCTGTCGGG
CTCCGGCGCCGCTTACCTTGGGCG

gcu7

TTAGGCTGCAGACCAAACAATGCCGTATTTCAAAGTCATTCTTTCCGGGCGTGGCATTGATCTGCCGTTTGATGGCGATTCGGCCATTGGTTTC
TTCACCACCAGGCTTGTCCGTTCTATGGACCCAGCAAGCGCTGAATCCTTGGCCAAAGGCCTAGTTCAGGCAGAGTGGCTCCCCGGCGGCACC
TATGCAACGGTTAATCGCGGCTCGCAGCCCACCTTGTCTGTCGAGCGGTCGTCCCCCGTCGGCTGGCTGACGGGCACTTTCGGTCGCAAACCA
TCCGGCTACTCATTCTACCGCCATGAGGACTAGACTGTGGCCTAACAATTCATTCAAGCCGACGCCACTTCGCGGCGCGGCTTACTTCAGGCG

gcu16

TTATGAGTATCTGTGCCCTGAGAGTATGAAAGCAGAATGGAAGCTCTATTCGTAGTATGCATTTATTTGGTGGTAGCCGGCATAGCCATTCTTC
CAGCCGTATTGGTTTGGAGCTTAGCTGGCCTATGGCTCCCCGCTTGGTTTTCACTACCTCCTATTCGTATGGCTTTAATATGTTTGGCATTATCC
ATTGTTACGGCATTGCTATTTAGTCTTGAACTTCCGGGCGGTATGCTTTCAGTTGGGCCATCTATCGTAGTTCTGTCATTGACCTGGTCTTTACT
TCTACTTCCCGTACTTTGTCTAGTTAGGTATTACCGCTATGCCACAACAAAACAGTCATAACAATCTGTTTCAGTACATTCCGGCACCTTTGGT
GCCTCCATCGGACAGCCTTTACAGCGTCGCGCCTCCAAGGCTGCCGCTGAACAAGGGCG

gcu22

TTAGACCTTCAGGAGGTGATAGGTGAGTTACAGAAATAAAACGTACGTCATCTTTGATGGCGATGAAGACATGTGGGCATACCGATACATGC
GTGGCTGGAAAGCTAACGAAAATATTGATTTCAACTTCTTTGATGCTCATGATTTGAAGCCGCTTACAGATCGTGCAGGAGAAGATACGGTTA
AGAGGCGGCTGAGGGAAAGGCTTGGGGATACCAAGCAGGCCATAGTTCTTATTGGCGAAAAAACAAAAAATCTGTATCGATTTGTGCGCTGG
GAACTTGAAACATGCATGAACCTGGATATACCAATCATAGCTGTTAATTTAAATGGACAAAGAAGCCAGGACGAGAATCTTTGTCCTCCAATA
ATTCGTGATGAGTATGTAGTCCACATCCCGTTCAAGCTAAAAATAATTCAGTATGCCTTGGACAACTTTCCAGGAGAGTTCCATCGGCGCAAT
CTAAGCGATAAAGGACCAAGGCTATACAACGACTCCGTCTACAAGCAGCTGGGGATCGAATGAAAAACGACGAATACCCTGCGCTGTACTGC
GCCACCAACAAGGCATCAATGGATGCCCAAAACCAGTACTTAAAGTTCGTTAAAGTTTATTCTCTTCTTCTTATTGCTGCGGCAGGGTTGGGCG
TTTACGGAATAAATGAAAAAGCATCTGCGGTCGCAGCCGCCTTGCTCGTTATAGGATCTATATTCCTATCTGTAATGATGCTGCTGAGACGAG
ATGAAGATACATGGTATCGGGCACGCTCTGTCGCCGAATCCGTTAAAACAAGCTCTTGGCGTTTCATGATGAGGTGCGAGCCTTACGTTGACG
CCCCTGATGTAAGAGTAGTGAAGTCAAATTTCAGGACACGACTGAAAAGTATCTTGAGTGAGCATAAGGACTTGGCGGAGCATCTAGGAGGT
TCGGTGTCTGAACAAGAGCAGATTACCGACAAGATGTGCGAAGTAAGAAATCTATCGTGGGAGCAGAGGGCTGACTTTTATCGCACGCATAG
AATTGACGAGCAACGTTCTTGGTATGCAAACAAGTCTGCTTGGAACCGCAAAAAAGGAAGGCTGTGGTTTGCTGTTTTAATCGGCTGCCAAGC
TTTTGCCGTTTTGTTCCTAATTTTGCGTGTTGCTTATCCGGATTGGGGATATTGGCCTGCGGATGTATTCGTTGTTGCGGCTGGATCGGCTTTAA
CCTGGATACAGGTGAAACGGTTTAAGGAGCTAGCAGCTGCATATGGATTGACAGCGCACGAGATTGGAGTTGTGAGAGGTGAGCTAGAGCAA
ATCGACTCTGGAGAAAAGTTGGCGCAGTTTGTAGCTGATAGCGAGAACGCATTTTCTAGAGAGCACACTCAATGGCTAGCTCGCAAAGACTCT
ATATAGGGTCTAACAATGCGCTGCTGTCGGACAATTTTTCCGCTGCGCTCCAAAATTGCCGCAGAGCGCGGCG

gcu23

TTATGCAGTCCAATGTCTGAATTAAGGAACTAAAAATGAGCACACGCCAAATCCATGTATTCATCAGTCATGCATGGAAATATTCTGGCCATT
ATCAAACGCTTGCTGATTGGATTTTTAATCAGAATTGGAGCGTGGGGCAAGCATCTCTGGATTTTAGAAATTATTCGGTTCCTAAAGATGATCC
AATTCATGATGCGCCAAATGATAGGCAGCTTAGAGATGCGATTATGAGGCAGATATCCATGAGTCATGTGGTAGTTATTCCTACAGGGATGTA
CACCAACTACAGCAAATGGATCGCAAAAGAAATTGAAGGTTCGACTGGTTTTAACAAACCTATTCTTGCTGTTAACCCCTGGGGACAGCAAAG
AACGTCAAGCGTAGTTGCGAATGCGGCAGCAAAGATAGTCGGATGGAATAAGCAGTCAGTAGTAGATGGTATCTGGGAGTTATACAAATAGT
GAGTAAGTTAGAAATAACCTCAGATGAGCTACCGGGTCTGTACCAATCAGCAAATCAAGCATCTCTCAACGCACAGGATAACTACTTCAGAG
GCTTGCGATGGTATCTTATCCTTTTGGTATGCGCGGCATTTATTTCATATGCCATGCCGAGAGATGCATTGGGCGCGCTGCTGTCAGCGGGACT
TTTCCTAGTTACATTAGGAATTCTTATTTTCATTCGTGTACAGCGCCCCGATGACACATGGTATAACGGGCGGGCGGTTGCTGAATCAGTGAAA
ACAAGAAGCTGGCGGTGGATGATGAGGGCTGAGCCGTATGAAGACTGTGAGAGTATGGAAATTGTTGCTAAGCAGTTTATCAACGACCTGAA
GACAATCTTGGAGCAAAACAAAAGCCTTTCTCACTCGCTGCAATCAACAAGTGCCGCCAAAGACCCTATCTCGCAGACGATGAAGGATGTTC
GCTCAAGAAACGTTAAAGATCGCTTGTCGATCTACATCGATCAAAGAGTGCAGAATCAAGTTGAATGGTACTGGCACAAGGCTCGCTTTAACA
AGCGAAGGGCGCAACAGTGGTTTTGGGTCTCGGTGATTCTGCATGCGCTTGCCATTGCCATGTTATTGTATCGCATTAAGGATCCTTCTTTTTC
GCTTCCTGTAGAGGTTATTGCAACAGGTGCGGGGGCTGCATTAACTTGGCTTCAAGCGAAAAAGCACAACGAATTAAACTCTGCATATGCTTT
AACGGCGCATGAAATTGTATTAATTAAGGGCGAGTCGGATTCTGTCCACGACGAAAAGCAACTGTCTGAGTATGTAATTAATAGCGAGGCTG
CATTTTCGCGCGAGCACACTCAATGGGTCGCTCGAAAGGGCGATTAATGCATAACAATGCGCTGCTGTCGGACAAATTTTACGCTGCGCTCCA
AATTTGCCGCAGAGCGCGGCG

gcu24

TTATAGCTCAAGCAGCTCATCTCGAAACGTGAAGGTTTTATGGACGAAATAGAATTAGATTTTGGCGCGATAACCATAGATAACGCGACCTAT
AAGGGCGAAGGTTATAGATTTTATGAGGGCTTACTTGCTCAGATGCGCCAATTTAAAGAAAGCCCAGTTAAAGTTCTTCAAACCGATATAGTG
CACAATGAAGCGATCAATCATATCGGGCAAGAGATATCAAAAACCCGATCCTCGATTGAGCAGGCACTAAGGTCAGCAAATAAACAGCTAAA
GATAAAATCCGAAGTTATAGAAAATGCTCGAGCGTTGCTATCAGTTGATGGCAGCGAAGCTGAAATAGCGGAAGCGAGGCTGGAAAAATATT
ATGAATTTATTGGAGCTGAGAAGATCGATTCTGGAAGATATGCAGATCTATCTCGTCTTATGGAAATGTATTTTGCTACAGAAGCTCCTTTTGA
ATCCGGAAAGGACAAAAAAAATGAATTTCCAGATGCGATTGCACTATTAGCCCTTGAAGGTTGGGCTGAAGAAAATGAAATTAATATTATCG
CCGTAAGTCAAGATAAGGGCTGGAAGAACTTTTCTGAAGGTTCAGATAGGATTACGTTGGTATCCTCTCTAGCGGAAGCGCTAGAAAAGTTTC
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gcu29
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gcu32
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gcu59
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gcu89

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gcu92

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gcu113

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gcu114

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gcu119

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gcu126

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gcu128

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gcu135

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gcu142

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gcu149

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gcu163

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gcu167

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gcu167.2

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gcu182

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gcu189

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gcu199

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gcu217

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gcu223

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gcu228

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gcu232

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gcuN

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gcuP

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gcuQ

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gcuWGS1

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gcuWGS2

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gcuWGS10

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gcuWGS21

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