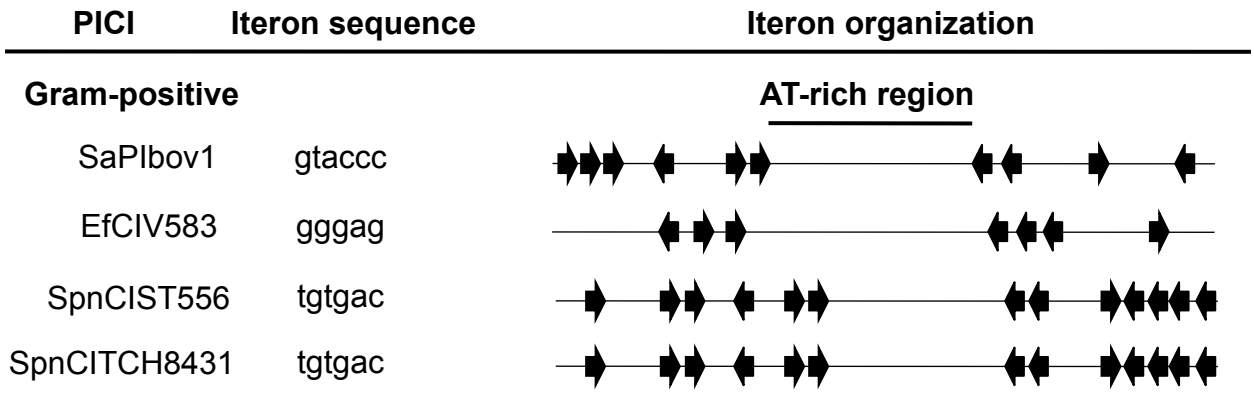
A. PICI replication origins



B. PICI autonomous replication

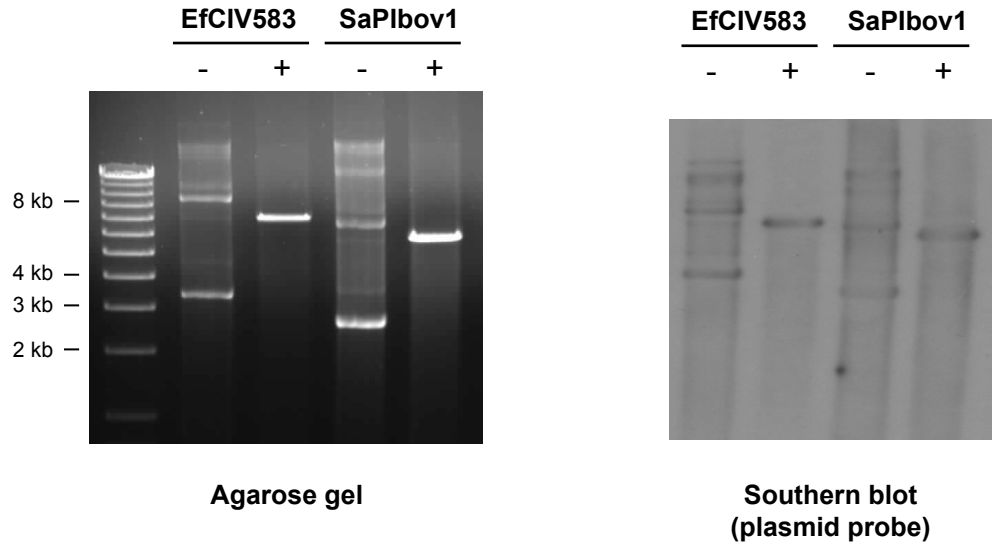


Figure S1. Characterization of the PICI replication origins. (A) Comparative map of the replication origins of several PICIs. The iterons are represented by arrows, and their sequences are shown at left. Note that there are always two sets of iterons flanking an AT-rich region, which could be the melting site. (B) Plasmid DNA carrying the Pri-Rep-*ori* segment of EfCIV583 or SaPIbov1 was isolated from overnight cultures of *S. aureus* RN4220, in presence of erythromycin. Plasmids were analyzed in agarose gels (left) or transferred for Southern blot studies using a probe specific for the plasmid (right). (-): non-digested plasmid; (+) plasmids digested with *Bam*HI, which cuts one in the plasmids.

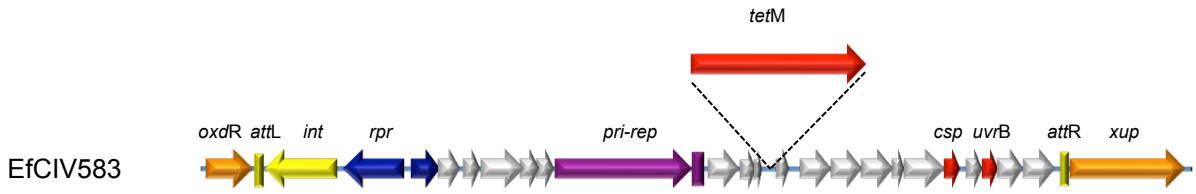
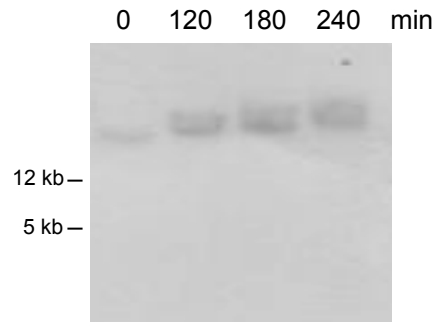


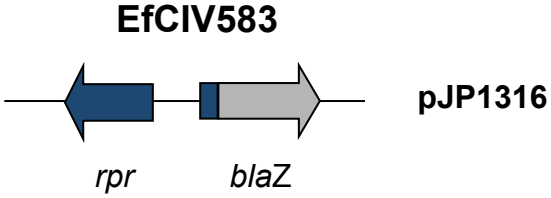
Figure S2. Location of the *tet*M marker in the EfCIV583 genome.

A

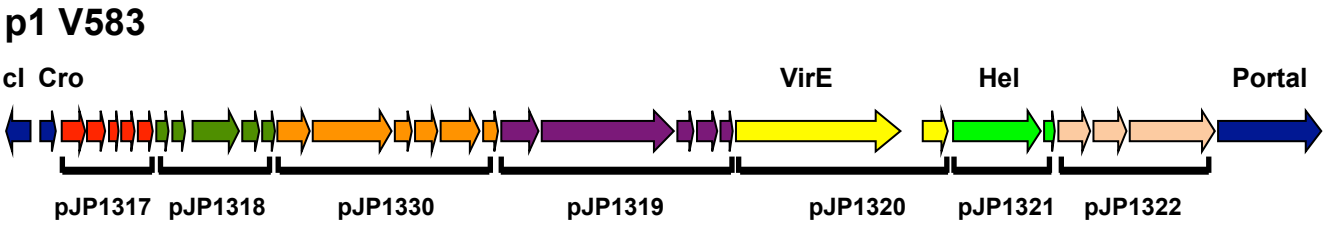


VE18589
EfCIV583, non lysogenic

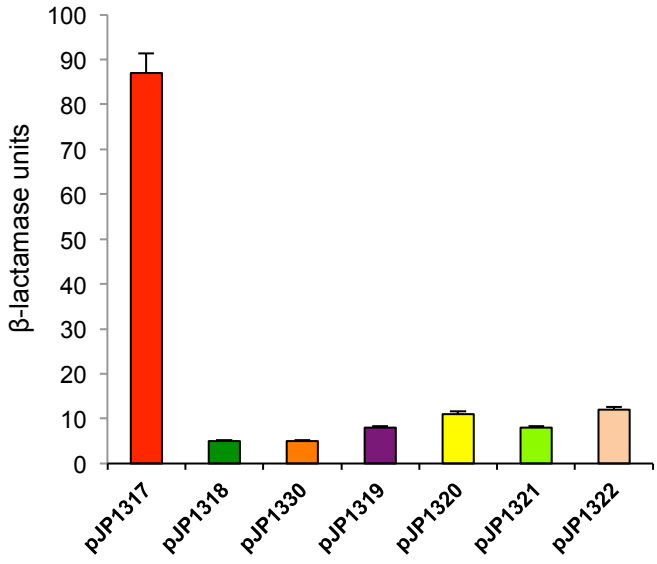
B



C



D



E

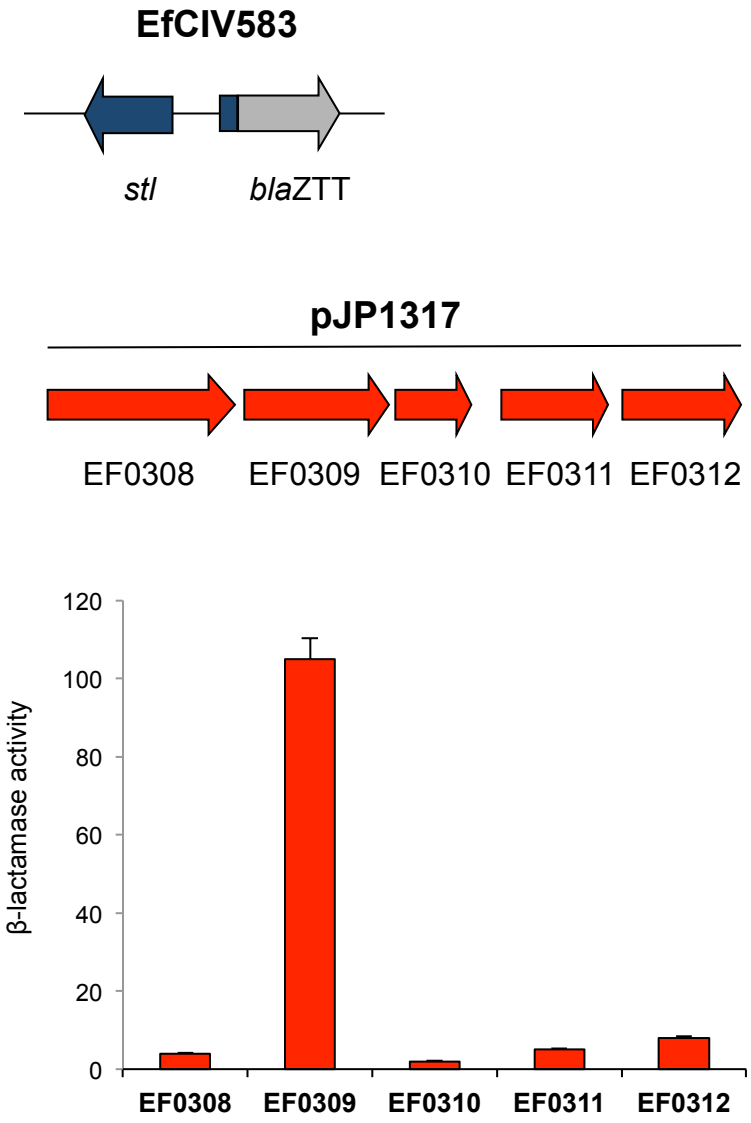


Figure S3. Identification of p1 region containing the derepressor of EfCIV583. (A) Failure of MC to induce excision/replication of EfCIV583. A culture of VE18589 (EfCIV538 positive) was induced with MC and samples taken during the subsequent incubation were used to prepare minilysates that were separated on agarose and Southern blotted with a EfCIV538 probe. (B) Construct used to test for de-repression. (C) Map of p1 showing regions that were cloned and tested for de-repression. *S. aureus* RN4220 derivative strains containing pJP1316 and pCN51 derivative plasmids (P*cad* promoter) containing the different regions of p1 were assayed for β-lactamase activity after induction with 5 μM $CdCl_2$. Samples were normalized for total cell mass. (D) β-lactamase activity generated by the above reporter construct in the presence of each of the cloned p1 regions, following induction with 5 um $CdCl_2$. (E) Demonstration of the de-repression activity of a subclone of pJP1317. Tests were performed with the construct shown in B. Values presented are the averages (±SD) of three independent assays.

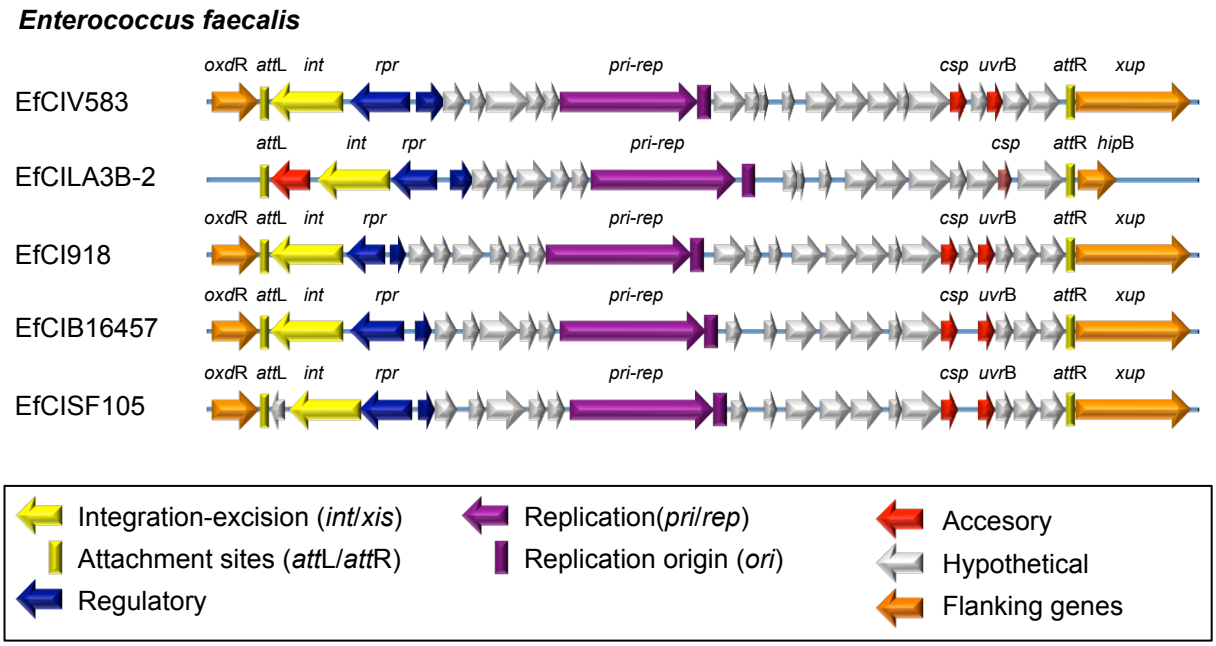


Figure S4. *Enterococcus faecalis* PICI genomes.

Figure S5A.
LICI-SK11 at *cut C* site, plus empty *cutC* site in strain NCDO

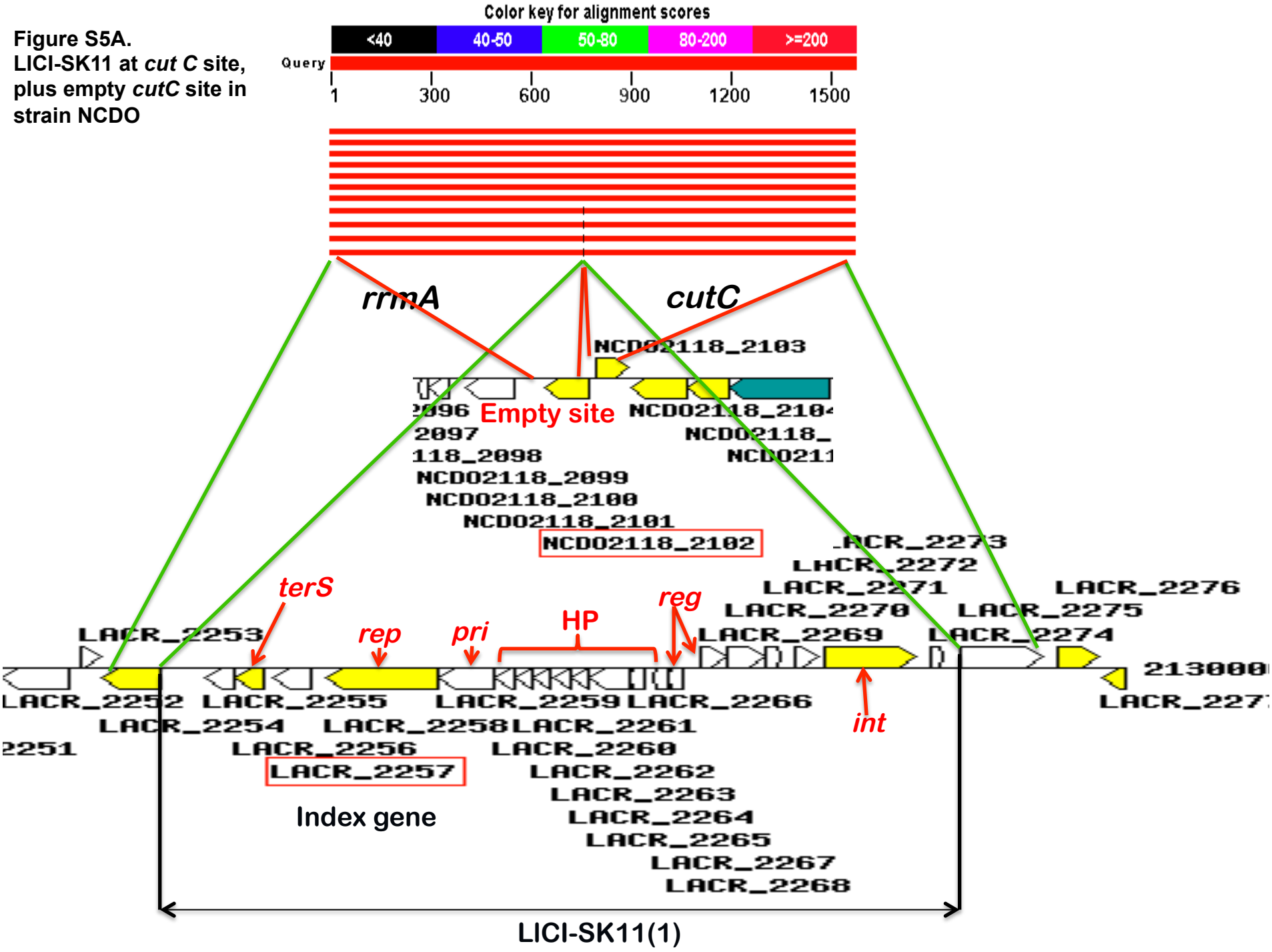
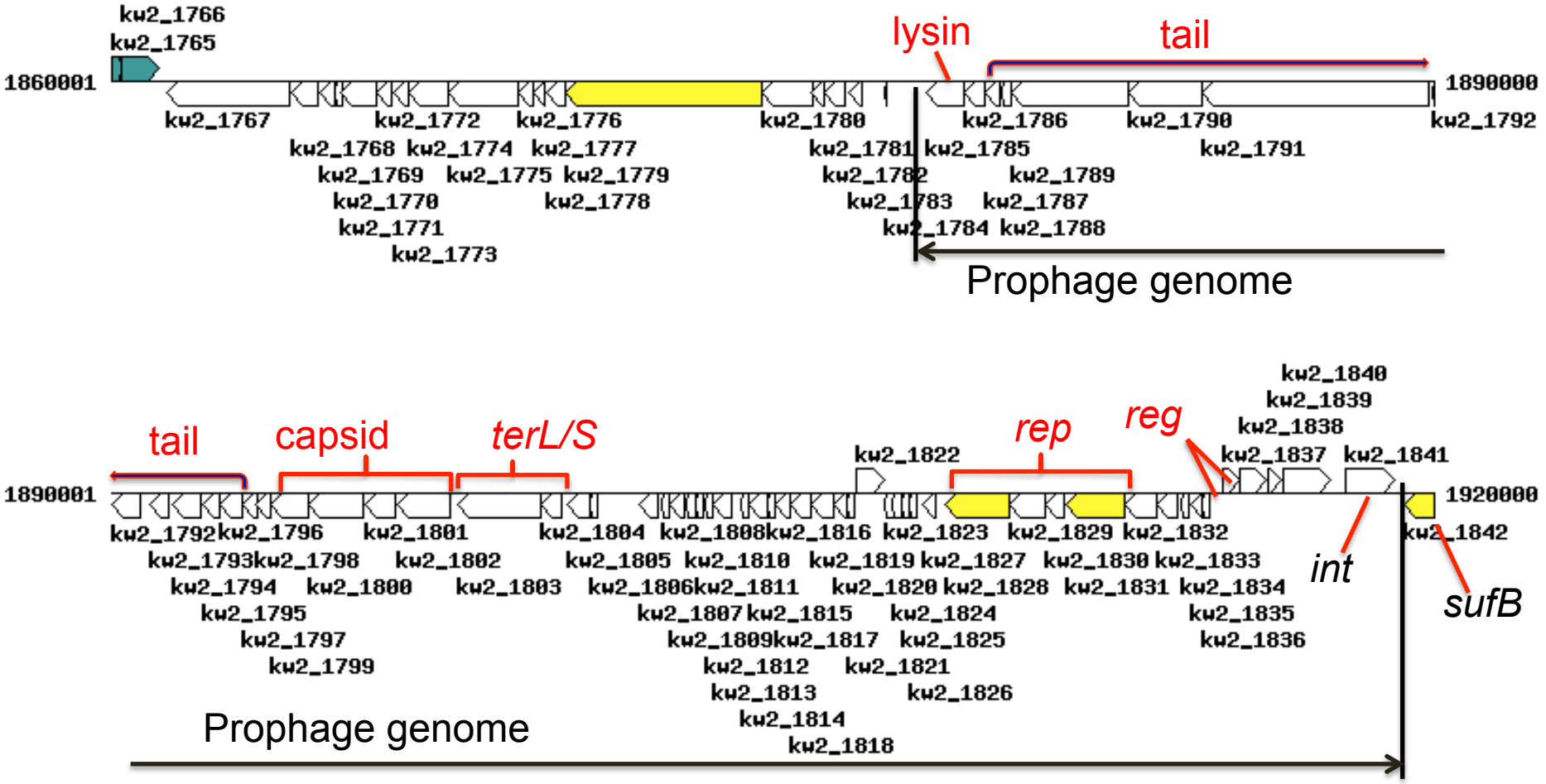


Figure S5B. LIPH-KW2 (Prophage) at *suf*B site



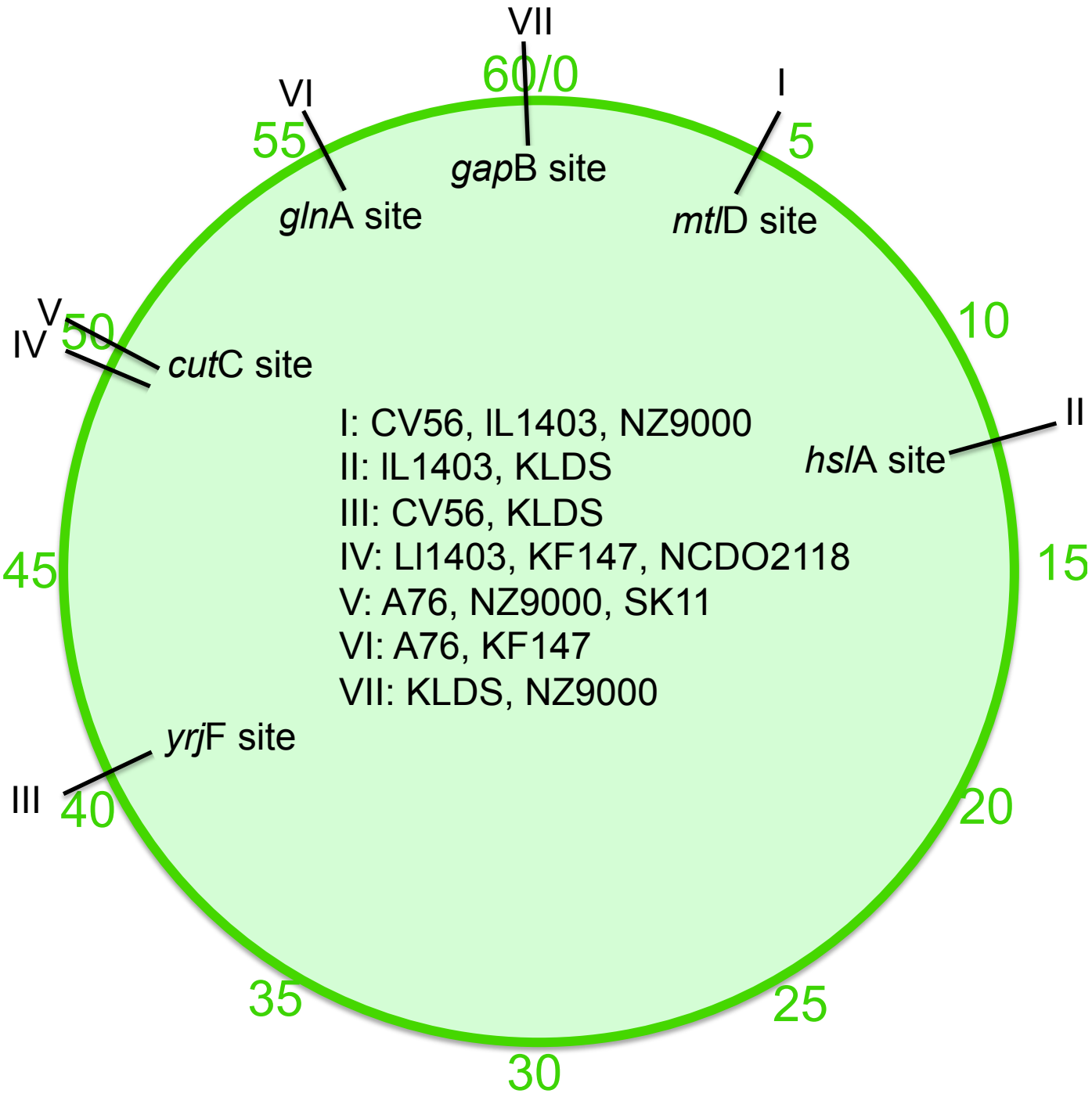


Figure S6. Locations of PICI *att* sites in lactococci.

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Phage bIL286    TTTTAATAACCCTCCCCCCGTATCTTTTCACAGGGAAACCACACACATAGGTATCGTCTT
LlCI-bIL310     TTTTTAAACACCCCCGCCCGTATCTTTTCACAGGGAAACCACACACATAGGTATCATCTT
                ****:*:*..** ** ****************************************.****

Phage bIL286    GCGTGAAAACCCATTTTTCAAAATTTTTATATAGGGGGGGTCAAAACACTAAAA
LlCI-bIL310     GCGTGAAAGTCCATTTTTGAAAATTTTTATATAGG-GGGGTCAAAAATCTAAAA
                ********. ******** *************** **********.:******

Phage bIL285    AAAGAACCGAGTGAGTTTAGCTTTTTCCAAGTGTGAGGAAATTTGAAAATATTTTTTTAC
LlCINZ9000-2    AAAGAACCGAGTGAGTTTAGCTTTTTCCAAGTGTACAAAGTCCTGAATCTATTTTTTTAC
                **********************************....*.:  ****:.***********

Phage bIL309    CCCCCCGTCATCGCTTTTAGGAATACCGTATACCAATGGTGGCTTCCTGAGTAAAAAAGT
LlCI-A76-2      CCCCCCGTCATTGCTTTTAGGAATACCGTATACCAATAGTGGCTCCCTGAGTAAAAAAGT
                *********** *************************.****** ***************

Phage bIL309    GATTTTTTAAAATTTTTGCATAGGGGGGGT
LlCI-A76-2      GGTTTTTTAAAATTTTTGCATAGGGGGGGT
                *.****************************
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Figure S7. Cos alignment. The predicted latococcal PICI and phage *cos* sites and their flanking sequences are aligned using ClustalW2.