SI Methods

Construction of plasmids

Plasmids pBK2Z and pBK2idtZ contain the gene *prgZ* preceded by the strong constitutive promoter P23 (P_{23}). This fragment is located upstream of prgX/prgQ in the same sense as *prgX*. The P_{23} sequence was amplified from plasmid pDL278p23[1] using primers p23-PstI-F / p23-PstI-R. A *Kpn*I recognition sequence was also included in p23-PstI-R. P23 was cloned into the *Pst*I sites of pBK2 and pBK2IdT. After sequencing confirmation, prgZ sequence was amplified from pCF10 using primers prgZ-KpnI-F / prgZ-KpnI-R, and was cloned into the *Kpn*I sites of pBK2P23 and pBK2ItTP23. The correct orientation of prgZ was verified by sequencing analysis.

Plasmids pBK2idTZ:Bac and pBK1:Bac, pBK2idTZ:EntA, pBK2idtZ:HirJM79 and pBK2idTZ:EntP were obtained by exchanging *lacZ* in pBK2idTZ and pBK1 by Bac, EntA, HirJM79 and EntP, respectively, using *BamH*I and *EcoR*I sites. Fragment Bac was amplified from plasmid pBac using primers prg:usp45-F / EntiP-R. Fragment EntA was amplified from plasmid pBac using primers prg:usp45-F / EntiA-R. Fragment HirJM79 was amplified from plasmid pBac using primers prg:HirJM79-F / HiriJM79-R. Fragment EntP was amplified from plasmid pBac using primers prg:EntP-F / EntiP-R. The correct orientation of Bac, EntA, HirJM79 and EntP was verified by sequencing analysis.

Supplementary References

1. Chen Y, Staddon JH, Dunny GM (2007) Specificity determinants of conjugative DNA processing in the Enterococcus faecalis plasmid pCF10 and the Lactococcus lactis plasmid pRS01. *Mol Microbiol* 63(5): 1549-64.

Table S1. Primers used in this study.

Primers	Nucleotide sequence (5' - 3')	Amplification of fragment
p23-PstI-F	AAACTGCAGGAAAAGCCCTGACAACGC	P23
p23-PstI-R	AAACTGCAG GGTACCAACATCATTGTCATTCATATTTT	P23
prgZ-KpnI-F	GGGGTACCATCCCTTTTAAAGTTTATTT	PrgZ
prgZ-KpnI-R	GGGGTACCTTATAAAAACGCGACGAACTA	PrgZ
prg:usp45-F	CATAGGATCCAAAAGGAGGAGAAAACTACTATGAAAAAAAAGATTATCTCAGCTAT	Bac, EntA and Lys170
EntiA-R	AAGGAATTCTTATTAAAATTGAGATTTATCTCCATAATCTGC	EntA
prg:HirJM79-F	CATAGGATCCAAAAGGAGGAGAAAACTACTATGAAAAAGAAAGTATTAAAACATTG	HirJM79
HiriJM79-R	AAGGAATTCTTATTAACTCCAAATACCAATAGAAGCC	Bac and HirJM79
prg:EntP-F	CATAGGATCCAAAAGGAGGAGAAAACTACTATGAGAAAAAAATTATTTAGTTTAGC	EntP
EntiP-R	CGAATTCTTATCAAAGTCCCGACCATGC	EntP
prgQ-F	CAGTTCATGTATATGTTCCCCGC	Bac, EntA, HirJM79, EntP and Lys170
prgQ-R	CTTTTAAGCCGTCTGTACGTTCC	Bac, EntA, HirJM79, EntP and Lys170

Supplementary figures

Figure S1.

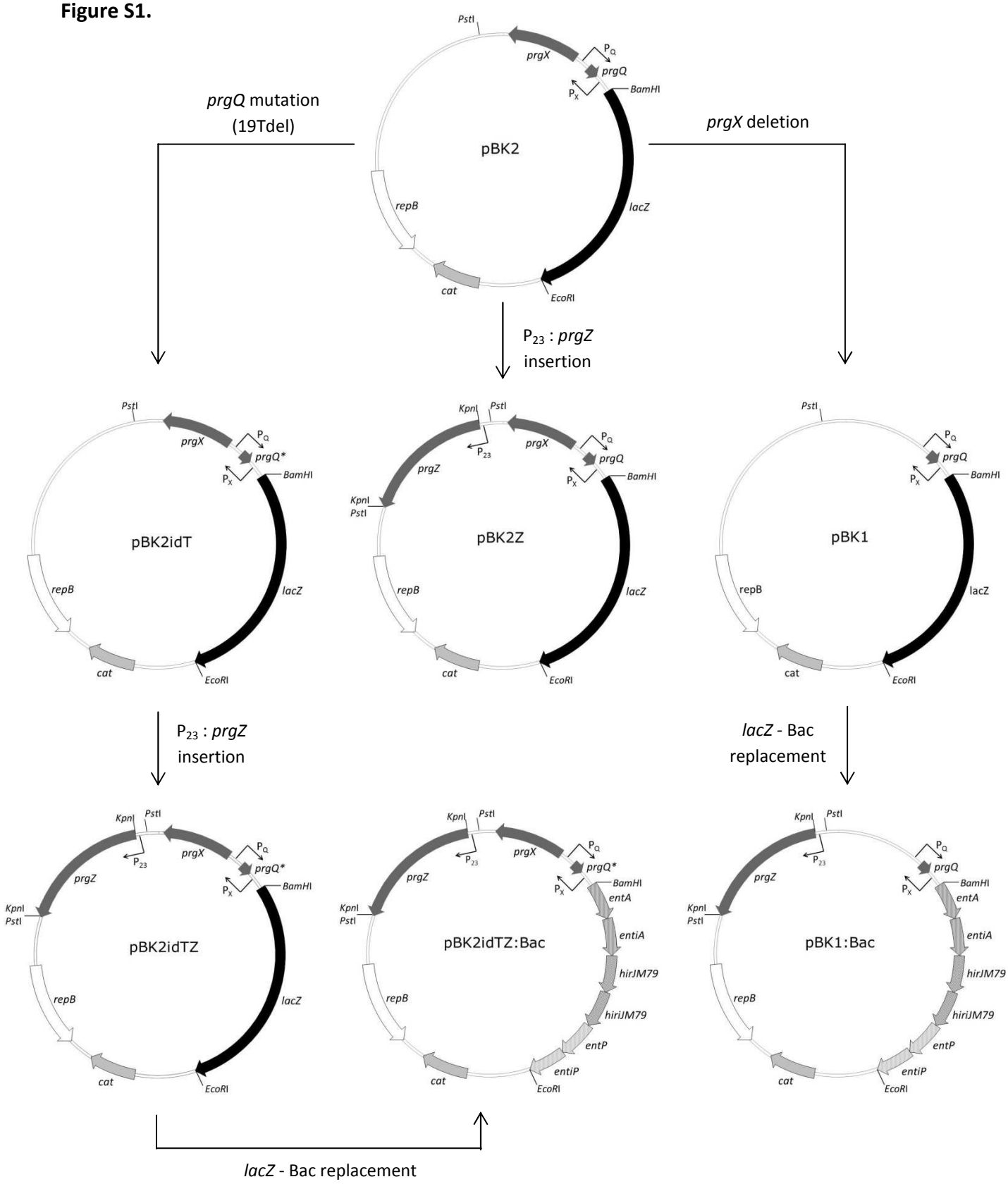

Figure S1. Construction of plasmids used in this work. pBK2, pBK2idT and pBK1 were prototype vectors previously constructed as discussed in Chen et al. (2007). *cat* is the chloramphenicol resistance marker. *repB* is the replication determinant. See Table 3 and text for sources and further description.

Figure S2.

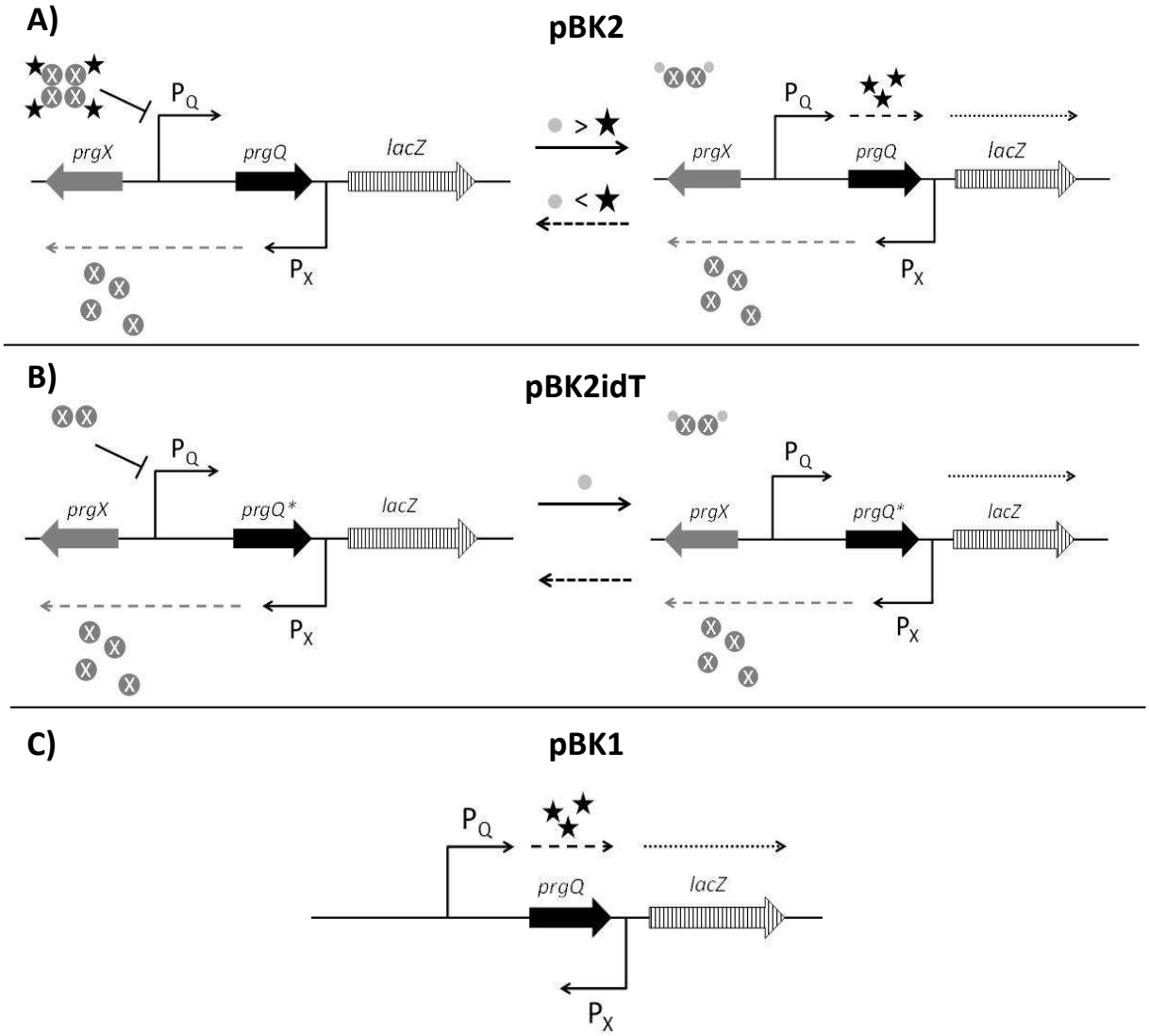

Figure S2. Three regulatory architectures cloned in lactic acid bacteria. A) pBK2 maintains the original pCF10 prgX/prgQ region intact. Both the inducer pheromone cCF10 and the repressor peptide iCF10 compete for binding to the master regulator protein PrgX. When the concentration of cCF10 is higher than that of iCF10, cCF10 binds to PrgX and the repression of P_Q stops triggering the expression of both *prgQ* and *lacZ*. When the concentration of iCF10 is higher enough than that of cCF10, iCF10 binds to PrgX, and the complex formed represses the expression of *prgQ* and *lacZ*. B) pBK2idT presents a mutation in *prgQ* (represented with and asterisk) producing a non-functional iCF10 protein. In the absence of inducer, PrgX forms a dimer that acts as a P_Q repressor. However when cCF10 is present, it binds to PrgX abolishing the repression of P_Q. C) pBK1 presents a deletion of *prgQ*. Under these circumstances there is no repression of P_Q, and the expression of *prgQ* and *lacZ* is always on, acting as a constitutive promoter.