Plasmid or strain	Relevant properties	Origin
Plasmids		
pCL52	Em ^R , a 2.4-kb <i>E. coli</i> cloning vector derived from	[1]
	pBluescript KSII	
PGh9	Em ^R , a 3.7-kb lactococcal <i>repA</i> ^{ts} cloning vector	[2]
pFC23	Cm ^R , pKO3-derivative plasmid (<i>repA</i> ^{ts}) containing the	[3]
	$rpsL^+$ gene. Used for replacement of the <i>E. coli dif</i> site by	
	allelic exchange	
pFX170	Ap ^R , full-length E. coli FtsK protein fused to a FLAG	[4]
	epitope on its N-terminus and cloned into pBAD24 vector	
pFX144	Cm ^R , pKO3-derivative (repA ^{ts}) plasmid. Used for allelic	[5]
	exchange at xerC locus in E. coli	
pCL231	Em ^R , pGh9-derivative (repA ^{ts}) plasmid. Contains a 37-bp	This work
	fragment, dif-2 (5'-TAACATCTTTCCgAAAAACTgTAA	
	TTTTCTTgACAAT-3') cloned at the XmaI site (the 31-bp	
	lactococcal dif_{SL} site is indicated in bold)	
pCL235	Em ^R , pCL52-derivative plasmid. Contains the same 37-bp	This work
	synthetic fragment than pCL231	
pCL237	Em ^R , pCL52-derivative plasmid. Contains a 27-bp	This work
	lactococcal dif _{SL} variant, dif-8 (5'-CTTTCCgAAAAACT	
	gTAATTTTCTTgA-3') of pCL233 (see Table 2) cloned at	
	the XmaI site	
pCL263	Em ^R , 5.35-kb XbaI-ClaI fragment from pFX170 (containing	This work

the FLAG-tagged full-length ftsK gene of *E. coli*, the araBAD promoter, and the *araC* gene) cloned at the corresponding sites of pCL52

- pCL294 Cm^R, 2.04-kb *dif*-2-Km^R-*dif*-2 cassette cloned at the *Xmn*I This work site of pFC23. Used for introducing the cassette at the *E. coli dif* locus
- pCL297 Cm^R, lactococcal *xerS* ORF (*ymfD*) cloned into one *Nco*I site This work of pFX144. *xerS* is expressed from transcriptional readthrough of the upstream *cat* gene
- pCL403 Em^R, pGh9-derivative ($repA^{ts}$) plasmid. Contains a 37-bp This work fragment (5'-TAACATCTTTCCgAAAAACTATAATTT TCTTgAAAAA-3') cloned at the *Xma*I site (the 31-bp pneumococcal dif_{SL} site is indicated in bold)
- pKNtergfp Em^{R} , 181 first amino acids of the lactococcal FtsK protein N. Campo, laboratory fused to Gfpmut1 and expressed from P_{nisA} of pNG8048e collection vector
- $pKFLgfp \qquad Em^{R}, full-length \ lactococcal \ FtsK \ protein \ fused \ to \ Gfpmut1 \qquad N. \ Campo, \ laboratory \\ and \ expressed \ from \ P_{nisA} \ of \ pNG8048e \ vector \qquad collection \qquad \\$

Strains

L. lactis

MG1363	Wild Type strain. Plasmid-free derivative strain of	[6]
	NCDO712	
VEL1122	Tc ^R , MG1363 <i>recA</i> :: <i>tetM</i>	[7]
NZ9000	MG1363 pepN::nisRK. Recipient strain for the nisin	[8]
	expression system.	

S. pneumoniae

R800	Wild Type strain. Derivative of strain R6	[9]
S501	R800 xerS (spr1046::Km)	This work
S502	R800 <i>ftsK</i> _C (spr0781:: <i>Km</i>)	This work
E. coli		
LN2772	Str ^R , Tc ^R , W1485 <i>leu thyA deoB</i> or <i>C supE rpsL</i>	[3]
	$\Delta(dif)$ 58::Tc	
LN3038	Str ^R , Tc ^R , W1485 <i>leu thyA deoB</i> or <i>C supE rpsL lacZ::Tn</i> 10.	F. Cornet, laboratory
	This strain has a wild type phenotype for XerCD/dif	collection
	recombination.	
E359	Str ^R , Km ^R , LN2772 with the lactococcal <i>dif</i> -2-Km ^R - <i>dif</i> -2	This work
	cassette inserted in the <i>tetA</i> gene	
E367	Str ^R , Tc ^R , Cm ^R , LN2772 containing pCL297	This work
E368	Str^{R} , Cm^{R} , E359 with one <i>dif</i> _{SL} site (the Km ^R cassette has	This work
	been excised by $XerS/dif_{SL}$ recombination) and containing	
	pCL297	
E372	Str^{R} , Km^{R} , Ap^{R} , E359 <i>ftsK</i> _C ::Ap	This work
E375	Str ^R , Tc ^R , Cm ^R , LN3038 containing pCL297	This work
E378	Str^{R} , Ap^{R} , Cm^{R} , E372 with one <i>dif</i> _{SL} site (the Km ^R cassette	This work
	has been excised by $XerS/dif_{SL}$ recombination) and	
	containing pCL297	
E379	Str ^R , Km ^R , Tc ^R , E359 <i>recA</i> 56	This work
E408	Str ^R , Km ^R , Ap ^R , Tc ^R , E359 <i>xerC</i> ::Ap	This work
E409	Str ^R , Km ^R , Ap ^R , Tc ^R , E359 <i>xerD</i> ::Ap	This work

References for Table S1

- Campo N, Daveran-Mingot M-L, Leenhouts KJ, Ritzenthaler P, Le Bourgeois P (2002) Cre/*loxP* recombination system for large genome rearrangements in *Lactococcus lactis*. Appl Environ Microbiol 68: 2359-2367.
- Maguin E, Prévots H, Ehrlich SD, Gruss A (1996) Efficient insertional mutagenesis in Lactococci and other Gram-positive bacteria. J Bacteriol 178: 931-935.
- 3. Cornet F, Mortier I, Patte J, Louarn JM (1994) Plasmid pSC101 harbors a recombination site, *psi*, which is able to resolve plasmid multimers and to substitute for the analogous chromosomal *Escherichia coli* site *dif*. J Bacteriol 176: 3188-3195.
- Yates J, Aroyo M, Sherratt DJ, Barre FX (2003) Species specificity in the activation of Xer recombination at *dif* by FtsK. Mol Microbiol 49: 241-249.
- Aussel L, Barre FX, Aroyo M, Stasiak A, Stasiak AZ et al. (2002) FtsK is a DNA motor protein that activates chromosome dimer resolution by switching the catalytic state of the XerC and XerD recombinases. Cell 108: 195-205.
- 6. Gasson MJ (1983) Plasmid complements of *Streptococcus lactis* NCDO712 and other lactic *Streptococci* after protoplast-induced curing. J Bacteriol 154: 1-9.
- Duwat P, Ehrlich SD, Gruss A (1995) The *recA* gene in *Lactococcus lactis*: characterization and involvement in oxydative and thermal stress. Mol Microbiol 17: 1121-1131.
- 8. Kuipers OP, de Ruyter PG, Kleerebezem M, de Vos WM (1998) Quorum sensingcontrolled gene expression in lactic acid bacteria. J Biotechnol 64: 15-21.

9. Lefevre JC, Claverys JP, Sicard AM (1979) Donor deoxyribonucleic acid length and marker effect in pneumococcal transformation. J Bacteriol 138: 80-86.