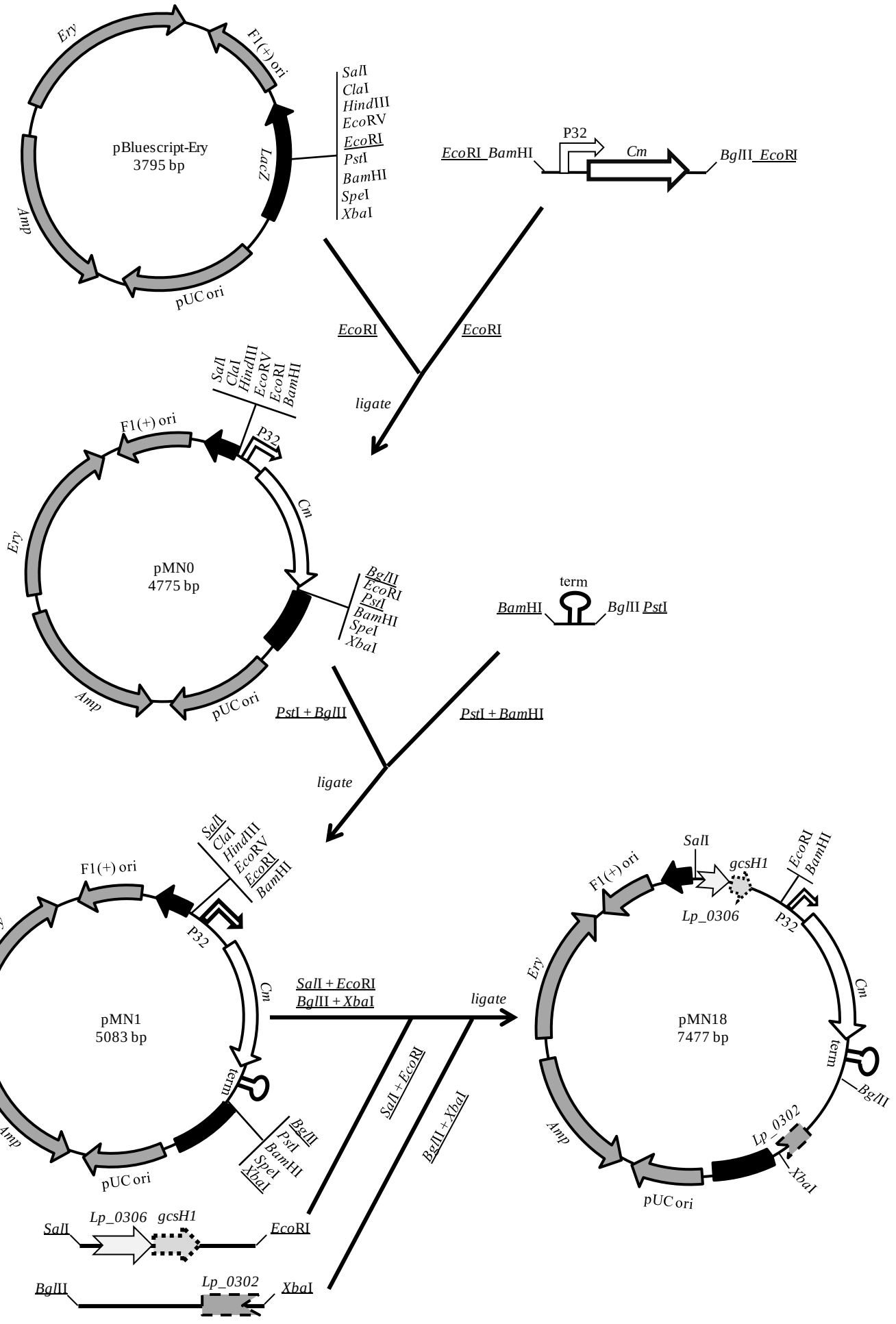
Suppl. Fig. 1


pBluescript-Ery
3795 bp
Ery
F1(+) ori
LacZ
Amp
pUC ori
SalI
ClaI
HindIII
EcoRV
EcoRI
PstI
BamHI
SpeI
XbaI
EcoRI BamHI
P32
Cm
BglII EcoRI
EcoRI
EcoRI
ligate
pMN0
4775 bp
BglII
EcoRI
PstI
BamHI
SpeI
XbaI
term
BamHI
BglII PstI
PstI + BglII
PstI + BamHI
ligate
pMN1
5083 bp
SalI + EcoRI
BglII + XbaI
ligate
SalI + EcoRI
BglII + XbaI
Lp_0306
gcsH1
Lp_0302
pMN18
7477 bp

Supplementary Material

Supplementary Figure 1. Construction of suicide vectors which led to plasmid pMN18, used in this work. Key features, replication origins, lengths and direction of the open reading frames and other structures are indicated. Only relevant restriction enzyme sites are shown. Molecules are proportional but not drawn to scale. P32-cat was amplified with the inclusion of *Eco*RI sites from plasmid pGIZ850. The transcriptional terminator region of *pepN* gene was amplified from *Lc. lactis* MG1363; in this step *Bam*HI and *Pst*I restriction sites were added. Two fragments containing i) 1,000 bp upstream and ii) 1,000 bp downstream of the gene coding for D1 protein (GI:342240594) were amplified with the addition of adequate restriction sites from *Lb. plantarum* NCIMB 8826.

pMN1 construction

pMN1 is a suicide vector that was conceived to generate and easily select gene knockouts in Gram positive bacteria. The insertion of a quimera chloramphenicol resistance gene with a strong promoter (P32; (Goffin, *et al.*, 2004) and a strong transcriptional terminator (from PepN; *(*Tan, *et al.,* 1992*)*, allows its selection in monocopy and avoids polar effects such as read-through into the adjacent genes. This resistance gene is flanked by two polylinkers in order to facilitate cloning the adjacent regions of the target sequence. An erythromycin resistance gene is included which allows differentiation of plasmid excision after a double crossover event. Moreover, the construction of clean knockouts (antibiotic resistance gene free) in a second step is also feasible because the chloramphenicol resistance gene is flanked by restriction sites *Bam*HI and *Bgl*II, allowing removal of that region by enzymatic treatment. Subsequent

ligation and electroporation with the resultant vector allows selection of clean mutants that would be sensitive to chloramphenicol.

Escherichia coli DH10B (Invitrogen) was used in all the cloning steps for plasmid construction. P32-cat was inserted into *Eco*RI site of a pBluescript II KS (+) (Agilent) derivative plasmid carrying erythromycin resistance gen from pUC19E (Leenhouts, *et al.*, 1990). *E. coli* DH10B transformants were selected in LB broth supplemented with ampicillin 100 µg/ml. Plasmids were checked and intermediate construction pMN0 was chosen. Primers TERM *Bam*HI and TERM *Pst*I/*Bgl*II were designed to amplify the transcriptional terminator region of *pepN* gene in *Lactococcus lactis* MG1363 (AM 406671; region 306556bp-306866bp), *Bam*HI and *Pst*I restriction sites were added in order to introduce it into the correct transcription orientation (Table 2). Total DNA of *Lc. lactis* MG1363, extracted with the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich), was used as the template and amplified with Pwo DNA Polimerase (Roche). Resulting amplicon was cut with *Bam*HI and *Pst*I (Fermentas) and ligated in pMN0 digested with the same enzymes using T4 DNA Ligase (Invitrogene) and electroporated in *E. coli* DH10B. Transformants were selected in LB supplemented with ampicillin 100 µg/ml. Plasmid DNA from a single isolate was sequenced (Macrogen). *E. coli* plasmid pMN1 were used to construct the suicide vector pMN18 for homologous recombination events in *Lactobacillus plantarum* NCIMB 8826 (see materials and methods).

References

Goffin P, Lorquet F, Kleerebezem M & Hols P (2004) Major role of NAD-dependent lactate dehydrogenases in aerobic lactate utilization in Lactobacillus plantarum during early stationary phase. *J Bacteriol* **186**: 6661-6666.

Leenhouts KJ, Kok J & Venema G (1990) Stability of Integrated Plasmids in the Chromosome of Lactococcus-Lactis. *Appl Environ Microbiol* **56**: 2726-2735.

Tan PST, Vanalenboerrigter IJ, Poolman B, Siezen RJ, Devos WM & Konings WN (1992) Characterization of the Lactococcus-Lactis Pepn Gene Encoding an Aminopeptidase Homologous to Mammalian Aminopeptidase-N. *FEBS Lett* **306**: 9-16.