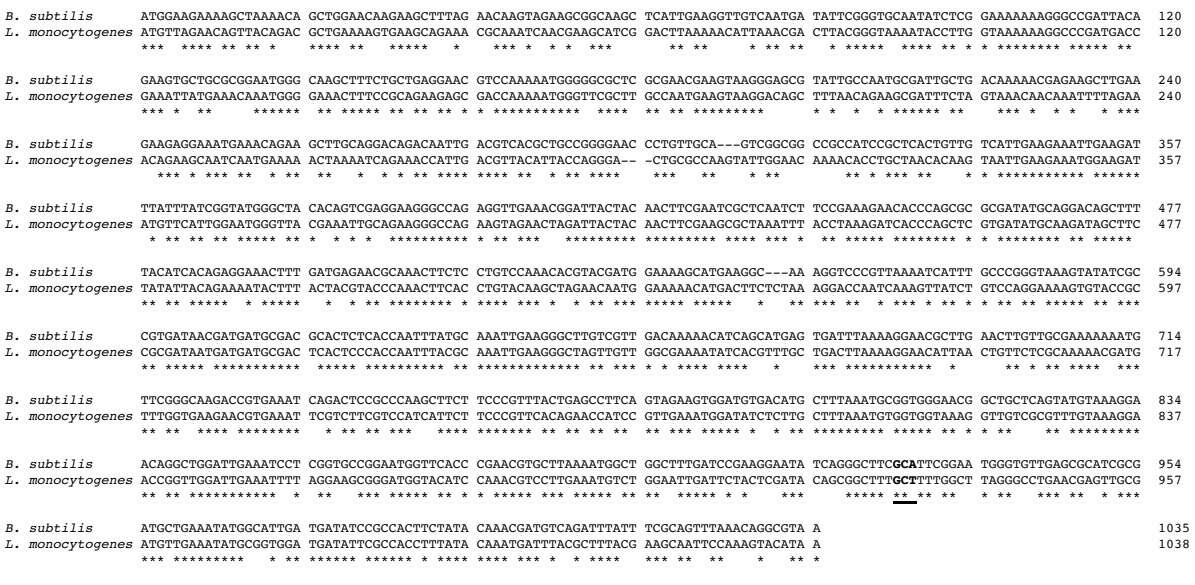
Figure S1



Percent DNA identity – 68.61%
Longest homology streak – 18bp

Multiple sequence alignment of *pheS* genes from *Listeria monocytogenes* 10403S and *Bacillus subtilis* 168.

Marked in bold and underscore are the codons for A309 (*B. subtilis*) A310 (*L. monocytogenes*). The Cytosine in the second position of the marked codon was mutated to Guanine, rendering the gene mutated, *pheS** (A309G) (materials and methods).

Alignment was generated by MUSCLE.

http://www.ebi.ac.uk/Tools/msa/muscle/

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res **32**:1792–1797.

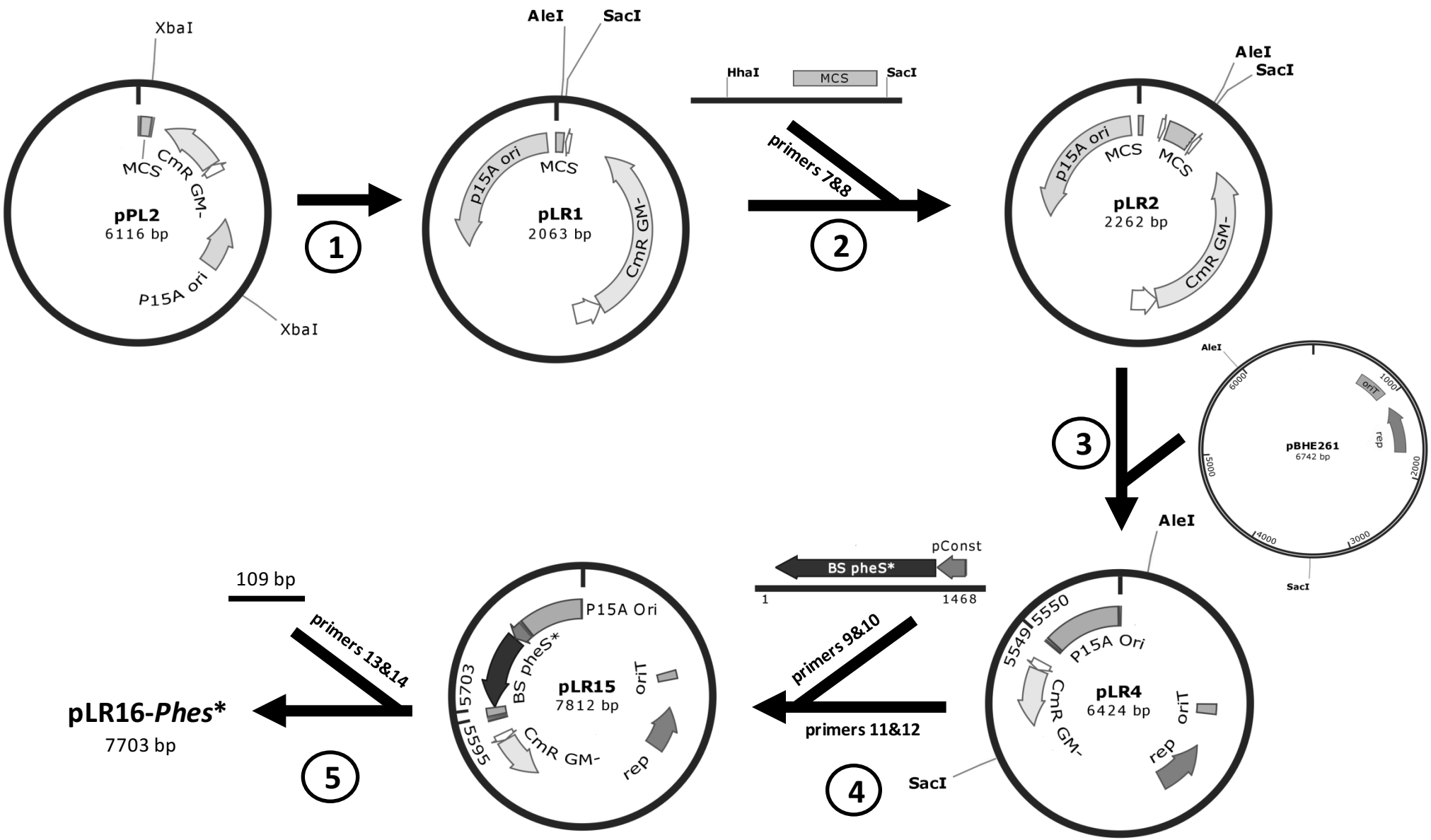

XbaI
MCS
CmR GM-
pPL2
6116 bp
P15A ori
XbaI
1
AleI
SacI
p15A ori
MCS
pLR1
2063 bp
CmR GM-
HhaI
MCS
SacI
primers 7&8
2
AleI
SacI
p15A ori
MCS
MCS
pLR2
2262 bp
CmR GM-
3
AleI
6000
1000
oriT
rep
pBHE261
6742 bp
5000
2000
4000
3000
SacI
AleI
pConst
BS pheS*
1
1468
primers 9&10
primers 11&12
4
5549
5550
P15A Ori
CmR GM-
pLR4
6424 bp
oriT
rep
SacI
109 bp
primers 13&14
pLR16-*Phes**
7703 bp
5
5703
5595
P15A Ori
BS pheS*
pLR15
7812 bp
oriT
CmR GM-
rep

Fig S2: PLR16-*pheS cloning strategy.** pLR16-*pheS** was constructed from pPL2 and pBHE261 (pKSV7oriT) plasmids. Step 1: pPL2 was digested with XbaI, and the fragment containing the P15A Ori and the *cat* gene conveying Gram negative Cm resistance (CmR GM-) was circularized to generate pLR1. Step 2: The multiple cloning site (MCS) of pPL2 was amplified using primers 7&8 (Table 1), digested with HhaI and SacI and ligated to pLR1 digested with AleI and SacI to generate pLR2. Step 3: AleI and SacI digested pLR2 and pBHE261 (the fragment containing the Ori for Gram positive bacteria, the origin of transfer (OriT), a replication gene (rep), gene for conjugative mobilization and a gene conveying Cm resistance to Gram positive bacteria) were ligated to generate pLR4. Step 4: pConst-*pheS** was amplified from pPL2-pConst-*pheS** (this study) using primers 9&10 (Table 1), and Gibson-assembled with pLR4 linearized at position 5549 using primers 11&12 (Table 1), to generate pLR15. Step 5: To generate the final 7703bp pLR16-*pheS** plasmid, a 109 bp sequence starting at position 5595 of pLR15 was removed by linearizing pLR15 using primers 13&14 (Table 1) and ligating the product.