

**Figure S1**

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B. subtilis      ATGGAAGAAAAGCTAAAACA GCTGGAAACAAGAAGCTTTAG AACAAAGTAGAAGCGGCAAGC TCATTGAAGGTTGTCAATGA TATTCCGGGTGCAATATCTCG GAAAAAAGGGCCGATTACA 120
L. monocytogenes ATGTTAGAACAGTTACAGAC GCTGAAAAGTGAAGCAGAAA CGCAAATCAACGAAGCATCG GACTTAAAAACATTTAAACGA CTTACGGGTAAAATACCTTG GTAAAAAAGGCCGATGACC 120
*** **
B. subtilis      GAAGTGCTGCCCGGAATGGG CAAGCTTTTCTGCTGAGGAAC GTCCAAAAATGGGGCGCTC GCGAACGAAGTAAGGGAGCG TATTGCCAATGCGATTGCTG AAAAAACGAGAAGCTTGAA 240
L. monocytogenes GAAATTAAGAAAACAATGGG GAAACTTTCCCGAGAAGAC GACCAAAAATGGGTTTCGCTT GCCAATGAAGTAAGGACAGC TTAAACAGAAGCGATTCTA GTAAACAACAATTTTAGAA 240
*** **
B. subtilis      GAAGAGGAATGAACAGAA GCTTGCAGGACAGACAATTG ACGTCAAGCTGCCGGGAAAC CCTGTTGCA---GTCGGGG CCGCCATCCGCTCACTGTTG TCATTGAAGAAATGAAGAT 357
L. monocytogenes ACAGAAGCAATCAATGAAA ACTAAAAATCAGAAACCAATTG ACGTTACATTACCAGGGA-- -CTGCGCCAAGTATTGGAAC AAAACACCTGCTAACACAAG TAATTGAAGAAATGAAGAT 357
*** **
B. subtilis      TTTATTTATCGGTATGGGCTA CACAGTGCAGGAAGGGCCAG AGGTGAAACGGATTACTAC AACTTCGAATCGCTCAATCT TCCGAAAGAACCCAGCGC GCGATATGCAGGACAGCTTT 477
L. monocytogenes ATGTTCAATGGAATGGGTTA CGAAATTCAGAAAGGGCCAG AAGTAGAACTAGATTACTAC AACTTCGAAGCGCTAAATTT ACCTAAAGATCACCAGCTC GTGATATGCAGATAGCTTC 477
*** **
B. subtilis      TACATCACAGGAAACTTT GATGAGAACGCAAACTTCTC CTGTCCAAACACGTACGATG GAAAGCATGAAGGC---AA AGGTCCCGTTAAAATCATTT GCCCGGTAAAGTATATCGC 594
L. monocytogenes TATATTACAGAAAATCTTT ACTACTGACCCAACTCTAC CTGTACAAGCTAGAAACAATG GAAAACATGACTTCTCTAA AGGACCAATCAAAGTTACT GTCCAGGAAAAGTACCCGC 597
*** **
B. subtilis      CGTGATAACGATGATGCGAC GCACTCTCAACAAATTTATGC AAATGGAAGGGCTTGTGTT GACAAAAACATCAGCATGAG TGATTTAAAAGGAACGCTTG AACTTGTGCGAAAAAATG 714
L. monocytogenes CCGGATAATGATGATGCGAC TCACTCCACCAATTTACGC AAATGGAAGGGCTAGTTGTT GCGAAAAATATCAGCTTTC TGACTTAAAAGGAACATTA CTGTTCTCGAAAAACGATG 717
*** **
B. subtilis      TTCGGGCAAGACCGTGAAT CAGACTCCGCGCAAGCTTCT TCCCGTTACTGAGCCTTCA GTAGAATGGATGTGACATG CTTTAAATCGCGTGGGAAC GCTGCTCAGTATGTAAAGGA 834
L. monocytogenes TTTGGTGAAGAACGTGAAT TCGCTTTCGTCCATCATCT TCCCGTTACAGAACCATCC GTTGAATGGATATCTCTTG CTTTAAATGTGGTAAAG GTTGTGCGGTTGTAAAGGA 837
*** **
B. subtilis      ACAGGCTGGATTGAATCCT CGGTGCCGGAATGGTTCACC CGAAGCTGCTTAAATGGCT GGCTTTGATCCGAAGGAATA TCAGGCTTCGCATTCCGAA TGGGTGTTGAGCGCATCGCG 954
L. monocytogenes ACCGGTTGGATTGAATTTT AGGAAGCGGATGGTACATC CAAACGTCTTGAATGTCT GGAATGATTTCTACTCGATA CAGCGCTTTCCTTTGGCT TAGGGCTGAACGAGTTGCG 957
*** **
B. subtilis      ATGCTGAATATGGCATTGA TGATATCCGCCACTTCTATA CAAACGATGTGACATTTATT TCGCAGTTTAAACAGGCGTA A 1035
L. monocytogenes ATGTTGAATATGCGGTGGA TGATATCCGCCACTTTATA CAAATGATTTACGCTTTACG AAGCAATTCCAAGTACATA A 1038
*** **

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

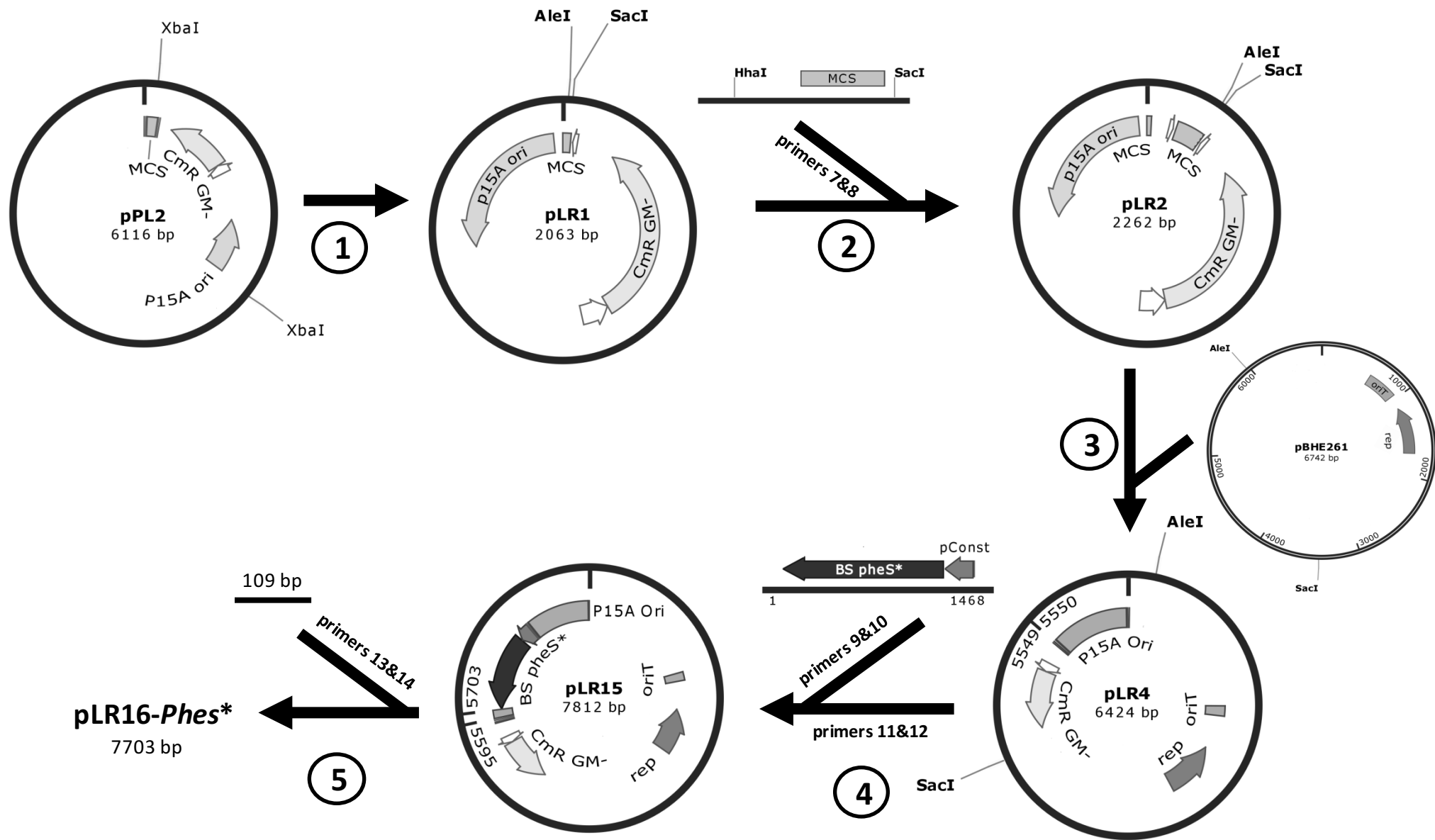
**Multiple sequence alignment of *pheS* genes from *Listeria monocytogenes* 10403S and *Bacillus subtilis* 168.**

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.



**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 AclI and SacI to generate pLR2. Step 3: AclI 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.