



## **Supplemental Material to:**

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**Exploring optimization parameters to increase ssDNA  
recombineering in *Lactococcus lactis* and *Lactobacillus  
reuteri***

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**Supplementary Table 1: Bacterial strains and plasmids used in this study**

Strain or plasmid	Characteristics <sup>†</sup>	Source <sup>*</sup>
<i>strains</i>		
<i>L. reuteri</i> ATCC PTA 6475	human breast milk isolate	Biogaia
<i>L. lactis</i> subsp. <i>cremoris</i> NZ9000	Derivative of MG1363, <i>pepN::nisRK</i>	1
<i>L. lactis</i> subsp. <i>cremoris</i> NZ9700	Nisin-producing strain	1
<i>L. casei</i> BL23	Human faecal isolate	2
<i>L. plantarum</i> BAA-793	Human oral isolate	3
<i>E. coli</i> DH5α		Invitrogen
<i>E. coli</i> SIMD44	<i>E. coli</i> derivative of HME6 [1 (int-cIII)< >EF2132*]	4
RPRB3007	Derivative of <i>L. reuteri</i> ATCC PTA 6475 in which CRE region upstream of <i>pdu</i> operon has been modified by incorporation of oJP675	
<i>plasmids</i>		
pJP005	Derivative of pNZ8048, cloned <i>recT1</i> under control of nisin-inducible promoter	5
pSIP411	Em <sup>R</sup> , Sakacin-P based expression vector	6
pJP042	Derivative of pSIP411, replaced <i>gusA</i> with <i>recT1</i> derived from <i>L. reuteri</i> ATCC PTA 6475	5
pJP043	Derivative of pSIP411, replaced <i>gusA</i> with <i>recT</i> derived <i>E. faecalis</i> CRMEN 19 (amplified from <i>E. coli</i> SIMD44)	This work
pJP045	Derivative of pSIP411, replaced <i>gusA</i> with <i>recT</i> derived from <i>L. casei</i> BL23 (accession number YP_001986912)	This work
pJP046	Derivative of pSIP411, replaced <i>gusA</i> with <i>recT2</i> derived from <i>L. casei</i> BL23 (accession number YP_001987247)	This work
pJP052	Derivative of pSIP411, replaced <i>gusA</i> with <i>recT</i> derived from <i>L. plantarum</i> BAA-793 (accession number YP_004888633 )	This work

<sup>†</sup>: CRE: Catabolite Repression Element; Em<sup>R</sup>: erythromycin resistant

**Supplementary Table 2: oligonucleotides used in this study**

oligo name <sup>§</sup>	sequence (5'-3') <sup>†</sup>	Target/comment <sup>#</sup>
<i>PCR</i>		
oJP161.1	<i>agcagctctagatcagaaaaggtaaggccgctc</i>	Rev oligo starting at stop codon <i>recT</i> gene <i>E. faecalis</i> CRMEN 19
oJP367	caatgatctagactcgaggaaatcggtacc	fwd oligo internal to XbaI site in pSIP411
oJP368	ttctacaggacgtaccatggctaaaatc	rev oligo internal to NcoI site in pSIP411
oJP371	catggctaaaatctccttgaatag	rev oligo starting at translational start site (ATG) located in NcoI site in pSIP411
oJP373	<i>agcagcccatggggaatgaattaatagtaagcgttc</i>	fwd oligo starting <i>recT</i> gene <i>E. faecalis</i> CRMEN 19
oJP409_PHO	gcgacaacatctttgcaaaaacaattaacg	fwd oligo starting at second codon <i>recT L. casei</i> BL23
oJP410_PHO	ctacttatctacgctgtcgtcgc	rev oligo starting at stop codon <i>recT L. casei</i> BL23
oJP411_PHO	acgacacaatatgacctaaaaaaatgccag	fwd oligo starting at second codon <i>recT L. casei</i> BL23
oJP412_PHO	ttatccttgtgaccgcaagattctg	rev oligo starting at stop codon <i>recT L. casei</i> BL23
oJP413_PHO	agtaatgagctagtacgatggttaataac	fwd oligo starting at second codon <i>recT L. plantarum</i> BAA-793
oJP414_PHO	ttagctggcgtcaaatctcgc	rev oligo starting at stop codon <i>recT L. plantarum</i> BAA-793
oJP415	agcatatatgtattctataaaatactattacaaggag	fwd oligo pSIP411 located upstream of MCS
oJP416	ttatcaactgctgcttttgctatcaatc	rev oligo pSIP411 located downstream of MCS
oJP673	taagacagctatgcagtcattgg	fwd oligo located upstream of CRE locus <i>pdu</i> operon
oJP674	tatctaatagttggatcaactctgc	rev oligo located downstream of CRE locus <i>pdu</i> operon
<i>Recombineering</i>		
oJP563	gagataccaccaggtcctaaggcagagaacgacgtttgtTGCTAagctcagacaaaggattatgttgctcataaattgt	<i>rpoB L. lactis</i>
oJP577	tcaaacaccaggaccaagcgtgaaagacgacgctTCTGCTtaattcacctaatgggtggttgatccatgaactgg	<i>rpoB L. reuteri</i>
oJP610	ccagttcatggatcaaaaccaaccattaggtgaattaaGCAGAAagcgtcgtcttcagcgtgctgctggtggtttga	leading strand <i>rpoB L. reuteri</i>
oJP675	gcctcctaagtataaaaaacaatagcgataatgagaaatcgGAATTCatttgggtacatgttaattttataacatgatatt	CRE locus <i>pdu</i> operon <i>L. reuteri</i>
oJP1147	<i>g*a*g*a*t*accaccaggtcctaaggcagagaacgacgtttgtTGCTAagctcagacaaaggattatgttgctcataaattgt</i>	<i>rpoB L. lactis</i>
oJP1148	<i>gagataccaccaggtcctaaggcagagaacgacgtttgtTGCTAagctcagacaaaggattatgttgctcataa*a*t*t*g*t</i>	<i>rpoB L. lactis</i>
oJP1149	<i>g*a*g*a*t*accaccaggtcctaaggcagagaacgacgtttgtTGCTAagctcagacaaaggattatgttgctcata*a*a*t*t*g*t</i>	<i>rpoB L. lactis</i>
oJP1157	acaattatggaccaacataatcttctgctgacttAGCAacaacgctgttctctgcttaggacctggtgctatctc	leading strand <i>rpoB L. lactis</i>
oJP1158	t*c*a*a*a*ccaccaggaccaagcgtgaaagacgacgctTCTGCTtaattcacctaatgggtggttgatccatgaactgg	<i>rpoB L. reuteri</i>
oJP1159	tcaaacaccaggaccaagcgtgaaagacgacgctTCTGCTtaattcacctaatgggtggttgatccatga*a*c*t*g*g	<i>rpoB L. reuteri</i>
oJP1160	t*c*a*a*a*ccaccaggaccaagcgtgaaagacgacgctTCTGCTtaattcacctaatgggtggttgatccatga*a*c*t*g*g	<i>rpoB L. reuteri</i>

§: \_PHO indicates oligonucleotides has phosphate group attached to the 5'-end of the oligonucleotide;

†: Italic sequence represents clamp; underlined sequence indicates restriction site; sequence in uppercase are the sequence changes for the recombineering oligonucleotide; \* indicates phosphorothioate linkage. With exception of oJP610 and oJP1157 are all recombineering oligonucleotides identical to the lagging strand of DNA replication. Upper case bases represent non-complementary bases.

#: *rpoB* DNA-directed RNA polymerase (locus tag HMPREF0536\_0828 for *L. reuteri*, LLNZ\_10235 for *L. lactis*). The locus tags listed can be found on <http://img.jgi.doe.gov/cgi-bin/w/main.cgi>; Fwd: forward; Rev: reverse; MCS: multiple cloning site; CRE: catabolite repression element. The accession numbers for each of the *recT* genes are listed in the Materials and Methods.

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