

Table S3.

Primer name	Primer sequence (5' – 3')	Description
Primer sequences used for lambda red mutagenesis*		
oMV442kan	TATTTCCGTAATATTCTCATTTGTCCTCGCCCCTGTT CTAACGTCCCATGTgttaggctggagctgcttc	<i>gtrC</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> and <i>S. Typhimurium gtrSTM_II</i> (GtrC Family IV) with kanamycin.
oMV444kan	ATTAGTCCCTTTTCGCGCGCTATTTCCGATGAAAAT GTAATCACTTTGCGattccgggatccgctgacc	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> and <i>S. Typhimurium gtrSTM_II</i> (GtrC Family IV) with kanamycin.
oMV728tetR	GACCTTTCCGAATCCGCTGATTTTCATAATGTTGAA GTTATTCGCTttaagaccactttcacatt	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Infantis gtrS1326/28_I</i> (GtrC Family V) with tetracycline.
oMV730tetA	GAATTATGGACAGGCTCCTTGTTGACTACTCTATAA CAATCCTTGCctaagcacttgtctcctg	<i>gtrC</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_I</i> (GtrC Family V) with tetracycline.
oMV731	AGGCTCCTTGTTGACTACTCTATAACAATCCTTGCA GCGAATAACTTCAACATTATGAAAATCAGCGGAT	Replacement of tetRA cassette from <i>S. Infantis gtrS1326/28_I::tetRA</i> (GtrC Family V)
oMV735tetR	ATTACAGTCTCTGAGGCGGAATTATAGTTAATTACA CCTTAGGttaagaccactttcacatt	<i>gtrC</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_II</i> (GtrC Family VI) with tetracycline.
oMV737tetA	AACATGCAAAGCCTTGCAAACCAATGCAAAGCTTT GTGTGTCCCgctaagcacttgtctcctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Infantis gtrS1326/28_II</i> (GtrC Family VI) with tetracycline.
oMV739	GCCTTGCAAACCAATGCAAAGCTTTGTGTGTCCC CTAAGTGGTGTAATTAATAATTCCGCCTCAG	Replacement of tetRA cassette from <i>S. Infantis gtrS1326/28_II::tetRA</i> (GtrC Family V)

oMV526tetR	GAACACCGATCGATGTGTACTTAGCGAATAACTTC AACATttaaagaccactttcacatt	<i>gtrA</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_II</i> regulatory region (GtrC Family VI) with tetracycline
oMV528pTac	GAACACCGATCGATGTGTACTTAGCGAATAACTTC AACATgactggctgttctctgtg	<i>gtrA</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_II</i> regulatory::tetRA (GtrC Family VI) with the pTac promoter.
oMV753pTac	AACATGCAAAGCCTTGCAAACCAATGCAAAGCTTT GTGTGTCCCGgacatcataacggttctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Infantis gtrS1326/28_II</i> regulatory::tetRA (GtrC Family VI) with the pTac promoter.
oMV531tetR	GCAGTATTAAGTACGCCTATCGAAACGTACTTTATA AATAATTTTATCATttaaagaccactttcacatt	<i>gtrA</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> regulatory region and <i>S. Typhimurium gtrSTM_II</i> (GtrC Family IV) with tetracycline.
oMV532tetA	GCTTCGCCTTATCCAGCCTGCAAAAGGTGCATAAA CACCGgtaagcactgtctctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> and <i>S. Typhimurium gtrSTM_II</i> regulatory region (GtrC Family IV) with tetracycline.
oMV533pTac	GCAGTATTAAGTACGCCTATCGAAACGTACTTTATA AATAATTTTATCATgactggctgttctctg	<i>gtrA</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> <i>S. Typhimurium gtrSTM_II</i> regulatory::tetRA (GtrC Family IV) with the pTac promoter.
oMV534pTac	GCTTCGCCTTATCCAGCCTGCAAAAGGTGCATAAA CACCGgacatcataacggttctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> <i>S. Typhimurium gtrSTM_II</i> regulatory::tetRA (GtrC Family IV) with the pTac promoter.
oMV764tetR	GACTCGATATAAATGGGCTAAGAGCTTTTGCATTGA TAAGTGTGTGCTGttaaagaccactttcacatt	<i>oafA</i> 5' primer for allelic replacement of <i>oafA</i> with tetracycline.
oMV765tetA	CCTGAGCCTTCTGGTGTAGGTGCGCATTGTCATAT TGTATAGGGTAAGCgtaagcactgtctctg	<i>oafA</i> 3' primer for allelic replacement of <i>oafA</i> with tetracycline.

oMV766	GGCTAAGAGCTTTTGCATTGATAAGTGTTGTGCTGG CTTACCCTATAACAATATGACAATGCGCACCTAAC	<i>oafA</i> primer for allelic replacement of <i>oafA</i> :tetRA.
oMV496tetR	TATTTCCGTAATATTCTCATTTGTCCTCGCCCCTGTT CTAACGTCCCATGTttaagaccactttcacatt	<i>gtrC</i> primer for allelic replacement of <i>S. Typhimurium gtrSTM_I</i> (GtrC Family III) with tetracycline.
oMV497tetA	TTGTCCCAAACACTTAGCAATCAGTAGCCCCAATT GATCGGTAACAACGctaagcactgtctcctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Typhimurium gtrSTM_I</i> (GtrC Family III) with tetracycline.
oMV498	CTCATTTGTCCTCGCCCCTGTTCTAACGTCCCATGTC GTTGTTACCGATCAATTGGGGCTACTGATTGCTA	Replacement of tetRA cassette from <i>S. Typhimurium gtrSTM_I::tetRA</i> (GtrC Family III)
oMV877tetR	AATTGCTGTAATTTACCTTTGTCTACAGAAGCGTA GTACCAGAATTCATttaagaccactttcacatt	<i>gtrC</i> primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> and <i>S. Typhimurium gtrSTM_II</i> (GtrC Family IV) with tetracycline.
oMV877tetA	ATTAGTCCCTTTTCGCGCGCTATTTCCGATGAAAAT GTAATCACTTTGCGctaagcactgtctcctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Infantis gtrS1326/28_III</i> and <i>S. Typhimurium gtrSTM_II</i> (GtrC Family IV) with tetracycline.
oMV878	ACCTTTGTCTACAGAAGCGTAGTACCAGAATTCATC GCAAAGTGATTACATTTTCATCGGAAATAGCGC	Replacement of tetRA cassette from <i>S. Infantis gtrS1326/28_III::tetRA</i> and <i>S. Typhimurium gtrSTM_II::tetRA</i> (GtrC Family IV).
oMV439kan	TATTTCCGTAATATTCTCATTTGTCCTCGCCCCTGTT CTAACGTCCCATGTgtgtaggetggagctgcttc	<i>gtrC</i> primer for allelic replacement of <i>S. Typhimurium gtrSTM_II</i> (GtrC Family III) with kanamycin.
oMV441kan	TTGTCCCAAACACTTAGCAATCAGTAGCCCCAATT GATCGGTAACAACGattccgggatccgctcgacc	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Typhimurium gtrSTM_II</i> (GtrC Family III) with kanamycin.
oMV526tetR	GAACACCGATCGATGTGTACTTAGCGAATAACTTC AACATttaagaccactttcacatt	<i>gtrA</i> primer for allelic replacement of <i>S. Typhimurium STM_II</i> regulatory region (GtrC Family III) with tetracycline.

oMV527tetA	CTTCGAAAAACATGCAAAGCCTTGCAAGCCGATGC AAAGCctaagcacttgtctcctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Typhimurium gtrSTM_II</i> (GtrC Family III) with tetracycline.
oMV528pTac	GAACACCGATCGATGTGTACTTAGCGAATAACTTC AACATgactggctgttctcctg	<i>gtrA</i> primer for allelic replacement of <i>S. Typhimurium gtrSTM_II</i> regulatory::tetRA (GtrC Family III) with the pTac promoter.
oMV529pTac	CTTCGAAAAACATGCAAAGCCTTGCAAGCCGATGC AAAGCgacatcataacggttctg	<i>gtr</i> regulatory region primer for allelic replacement of <i>S. Typhimurium gtrSTM_II</i> regulatory::tetRA (GtrC Family III) with the pTac promoter.

Primer sequences used for cloning and mutagenesis of *gtr* regulatory region- *lacZ* reporter fusion constructs (in order of strains Table 1)**

oMV412	catgatgtaccCTTCAACATTATGAAAATCAGCGG	R-primer regulatory region for generating <i>lacZ</i> fusion of sMV444, sMV445, sMV84, and sMV352; with Acc651 site
oMV846	ggtagctctgcagAACCTCGACTTAGAAGTTTCTG	F-primer Regulatory region for <i>lacZ</i> fusion sMV444 with PstI site
oMV848	ggtagctctgcagCCCGCGGCTTAGATGTTCTGG	F-primer Regulatory region for <i>lacZ</i> fusion sMV445 with PstI site
oMV458	catgatgtaccTTTTATCATTATAGCAATCA	R-primer regulatory region for generating <i>lacZ</i> fusion of sMV446 and sMV521; with Acc651 site
oMV459	ggtagctctgcagGCTTCGCCTTATCCAGCCTG	F-primer Regulatory region for <i>lacZ</i> fusion sMV446, with PstI site
oMV414	ttgattcctgcagCCCACGGCTTAGATGTTCTGG	F-primer Regulatory region for <i>lacZ</i> fusion sMV84 and sMV520 with PstI site

oMV504	AGCAATCAGTAGCCCCAATTCATCGGTAACAACGA	Mutagenic sense primer for GATC 1 mutation in regulatory region for <i>lacZ</i> fusion in sMV510
oMV505	TCGTTGTTACCGATGAATTGGGGCTACTGATTGCT	Mutagenic antisense primer for GATC 1 mutation in regulatory region for <i>lacZ</i> fusion in sMV511sMV510
oMV514	CAAACCACTTAGCAATCAGCAATAAAAATTCATCG GTAACAACGATC	Mutagenic sense primer for GATC 1 mutation in regulatory region for <i>lacZ</i> fusion in SMV511
oMV515	GATCGTTGTTACCGATGAATTTTTATTGCTGATTGC TAAGTGGTTTG	Mutagenic antisense primer for GATC 1 mutation in regulatory region for <i>lacZ</i> fusion in sMV511
oMV413	ggtagctctgcagCTTCGCATTACGAATTATAAGAAC	F-primer Regulatory region for <i>lacZ</i> fusion sMV517 with PstI site
oMV850	catgatggtaccTGTAACAACACTATGAGTCCTTGGA	R-primer regulatory region for generating <i>lacZ</i> fusion of sMV517, with Acc651 site
oMV852	ggtagctctgcagCAATCTATCTATTTTAACAATCAATTTA AG	F-primer Regulatory region for <i>lacZ</i> fusion sMV518 with PstI site
oMV851	ggtagctctgcagAGTCCACGGCTTAGAAGTTTCTGTAACC GCCG	F-primer Regulatory region for <i>lacZ</i> fusion sMV520 with PstI site
oMV459	ggtagctctgcagGCTTCGCCTTATCCAGCCTG	F-primer Regulatory region for <i>lacZ</i> fusion sMV521 with PstI site

* Lowercase primer sequences indicate homology to the amplified antibiotic marker used for knockout selection or the pTac promoter. Synonyms: Kan, kanamycin (as designed by [44]); tetR/A, tetracycline as designed by [46]. pTac: refers to the constitutive pTac promoter (116bp) [28] that was used for allelic replacement of the native *gtr* promoter.

** Lowercase primer sequences indicate sequence added to include restriction enzyme recognition sequence.