

Table S1. Plasmids and primers used in this study

Plasmid or primer	Description <sup>a</sup>	Reference or source <sup>b</sup>
<b>Plasmids</b>		
pFW13	<i>E. coli</i> -streptococcal shuttle vector; <i>E. coli</i> origin of replication and Kan <sup>r</sup> gene ( <i>aacA/aphD</i> )	[52]
pFWKR	Bacteriophage counter-selection vector; pFW13 + SF370 <i>rpsL</i> <sup>WT</sup> ( <i>Sm</i> <sup>r</sup> ) gene and promoter region inserted after Kan <sup>r</sup> gene to create the Phage KO Janus Cassette (( <i>aacA/aphD</i> ) / <i>rpsL</i> <sup>WT</sup> )	This Study
pFWKR-speC	Φ370.1 counter-selection vector; pFWKR with adjacent regions to <i>speC</i> inserted into MCS sites	This Study
pFWKR-speH	Φ370.2 counter-selection vector; pFWKR with adjacent regions to <i>speH</i> inserted into MCS sites	This Study
pFWKR-spd3	Φ370.3 counter-selection vector; pFWKR with adjacent regions to <i>spd3</i> inserted into MCS sites	This Study
pFWKR-2136	SpyCIM1 counter-selection vector; pFWKR with adjacent regions to <i>spy2136</i> inserted into MCS sites	This Study
Primers & Description	Sequence <sup>b,c</sup>	Product size
<b>Janus Cassette</b>		
<i>rpsL</i> <sup>WT</sup> gene + upstream promoter from SF370 to clone into pFW13		676
rpsLF1 (MabI)	AGGTTCC <b>ACCAGG</b> TATCTAAGTGATGCATTC	
rpsLR1 (EcoICRI)	TTACGACTCAT <b>GAGCTC</b> TTATCCCCCTTCTA	
Janus Cassette from pFWKR for PCR fusion rxns to adjacent regions in each bacteriophage		2344
kan/rpsLFwdFusion	<i>GCTTGATTTTCGTTTCGTGAATACATGTTATAATAAC</i>	
kan/rpsLRevFusion	<i>GGCGCGCTTACCAATTAGAATGAATATTTC</i>	
Kan <sup>r</sup> ( <i>aacA/aphD</i> ) gene from pFW13 (PCR amplicon used as Southern blot probe)		1431
kanFwd	ATGAATATAGTTGAAAAATGAAATATGTA	
kanRev	TTAAGTCCTCTTATAAAATTTCTTTTCT	
<b>Φ370.1</b>		
Upstream region adjacent to <i>speC</i> gene for PCR fusion to Janus Cassette		727
UpSpeCKoFwd-Fus	GACAATAACTGTTGACGCAGCTAGGGTACGAACCTTATCC	
UpSpeCKoRev-Fus	<i>GTTATTATAACATGTATTACGAACGAAATCAAGCTTAGTTTTAGGAGTGGCAGTTCATTAAATAGTCAAGGTTG</i>	
Downstream region adjacent to <i>speC</i> gene for PCR fusion to Janus Cassette		588
DoSpeCKoFwd-Fus	<i>GAAATATTCAATCTAATTGGTAAGCGCGCCCATCATACACAAAAACCGCCAGATAATCTGAGC</i>	
DoSpeCKoRev-Fus	CCAATCAGTGCCGTATCACTAATTTGGACATACG	
Nested PCR of Fusion PCR amplicon for cloning into pFWKR to construct pFWKR-speC		3561
NestNheI370.1 (NheI)	TTGACGCAGCTAGCGTACGAACCTTATCC	
NestXmaI370.1 (XmaI)	ATTTGGACAC <b>CCCGGGA</b> ATTGTTATCGAG	
Φ370.1 phage <i>speC</i> gene (PCR amplicon also used as Southern blot probe)		546
SpeCFwd	TATAAAAATTGCAGGGTAAA	
SpeCRev	ATAAATATCGAAATGACTAAG	
Φ370.1 phage <i>spd1</i> gene (PCR amplicon also used as Southern blot probe)		402
Spd1/MF2Fwd	CCCCTTCAGGATTGCTGTCAT	
Spd1/MF2Rev	ACTGTTGACGCAGCTAGG	
Across Φ370.1 phage <i>attP</i> -L junction		389
370.1spy0654Fwd	GCTCTTGAGTTCCCAATCACC	
370.1int1Rev	GACTCTCTTGGAATGCTGATGG	
Across Φ370.1 phage <i>attP</i> -R junction		1118
Spd1/MF2Fwd	CCCCTTCAGGATTGCTGTCAT	
370.1PepDRev	AACACGAGAATTGGTAGTGATGG	
Across Φ370.1 <i>attB</i> in phage KO (PCR amplicon also used as Southern blot probe)		519
370.1spy0654F	GCTCTTGAGTTCCCAATCACC	
370.1PepDRev	AACACGAGAATTGGTAGTGATGG	
<b>Φ370.2</b>		
Upstream region adjacent to <i>speH</i> gene for cloning into pFWKR to construct pFWKR-speH. From Ryan <i>et al.</i> [54] <sup>b</sup>		662
UpSpeHkoFwd (BamHI)	TCTACATAGGATCCAACAGAAGTAAAAAGG	
UpSpeHkoRev (HindIII)	TTTCTCCTTA <b>AAGCTTT</b> GAGAGTTTTATTTA	
Downstream region adjacent to <i>speH</i> gene for cloning into pFWKR to construct pFWKR-speH. From Ryan <i>et al.</i> [54] <sup>b</sup>		888
DoSpeHkoFwd (ApaI)	CATGTAGATGGGCCCATCAGCTAATTA	
DoSpeHkoRev (AvrII)	AAAAACCTAGGTATACCAGTCATGACCAGG	
Φ370.2 phage <i>speH</i> gene (PCR amplicon also used as Southern blot probe)		318
SpeHFwd	GTGAATGTCCAGGAAAAGG	
SpeHRev	GCATGCTATTAAGTCTCCATTG	
Across Φ370.2 phage <i>attP</i> -L junction		548
cpsFQPhageFWDattL	CTTATGATCACGTAACGTACCGTGC	
inteRevphageAttLRev	GCCCTCAACACCTCTATCAGAC	

<b>Across <math>\Phi</math>370.2 phage attP-R junction</b>		753
HKOFWDW/EXSEQR	CGACTTTGTAACCATTTATTGTTCC	
mutXR	GGTCACAGATTATCCATGAGTTG	
<b>Across <math>\Phi</math>370.2 attB in phage KO (PCR amplicon also used as Southern blot probe)</b>		906
cpsFQPhageFWDattL	CTTATGATCACGTAACGTACCCGTGC	
mutXR	GGTCACAGATTATCCATGAGTTG	
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<b><math>\Phi</math>370.3</b>		
<b>Upstream region adjacent to <i>spd3</i> gene for PCR fusion to Janus Cassette</b>		1058
UpSpd3KoFwd-Fus	TGAAGATGGTCTGCCATCCATTGTCTGATTGTC	
UpSpd3KoRev-Fus	GTTATTATAACATGTATTACGGAACGAAATCAAGCCTAAACCTTGCCAAGTACGACGATTGTATTAGACATATCC	
<b>Downstream region adjacent to <i>spd3</i> gene for PCR fusion to Janus Cassette</b>		1388
DoSpd3KoFwd-Fus	GAAATATTCAATTGTAAGCGCGCCATAAACTTATACTTAATAAAAATATCTAGATTGTGAACATTATCC	
DoSpd3KoRev-Fus	CGATTGACTTACTAGATAGCGCTGCTGC	
<b>Nested PCR of Fusion PCR amplicon for cloning into pFWKR to construct pFWKR-spd3</b>		4650
NestXmaI370.3 (XmaI)	TTTAGTTCCCGGGCAATTAAGATTATTC	
NestNheI370.3 (NheI)	GAAGTCCACCGCTAGCCGACAG	
<b><math>\Phi</math>370.3 phage <i>spd3/mf3</i> gene (PCR amplicon also used as Southern blot probe)</b>		482
spd3/MF3Fwd	CAAATTGACTGACGCTAATGG	
spd3/MF3Rev	CCGCTTCTTCAAACCTTCG	
<b>Across <math>\Phi</math>370.3 phage attP-L junction</b>		875
Spy1434out370.3JxnF	TCTTCACCAAATCCAATATACCC	
370.3ATTLjxntestR	CTAACATACGACGAGTTTAAGC	
<b>Across <math>\Phi</math>370.3 phage attP-R junction</b>		760
hlpASF370.3JXR	ATGGCTAACAAACAAGATTTAATCG	
int2SF370.3F	GACATTAGCTTCTTATCGTGAAACC	
<b>Across <math>\Phi</math>370.3 attB in Phage KO (PCR amplicon also used as Southern blot probe)</b>		806
Spy1434out370.3JxnF	TCTTCACCAAATCCAATATACCC	
hlpASF370.3JXR	ATGGCTAACAAACAAGATTTAATCG	
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<b>SpyCIM1</b>		
<b>Upstream region adjacent to <i>spy2136</i> gene for PCR fusion to Janus Cassette</b>		781
UpSpy2136KoFwd-Fus	CAGTTTAGACCTTTGAAACCGCCTGAGG	
UpSpy2136KoRev-Fus	GTTATTATAACATGTATTACGGAACGAAATCAAGCTGCTGG TAAACGTCCTATCTATCTCATCAATGG	
<b>Downstream region adjacent to <i>spy2136</i> gene for PCR fusion to Janus Cassette</b>		818
DoSpy2136KoFwd-Fus	GAAATATTCAATTGTAAGCGCGCCTTAGTCATTTTATACGTTTAGCATTTAAAAATTAACG	
DoSpy2136KoRev-Fus	GTGATAAGTTGCGTCAATCACTGGTTGC	
<b>Nested PCR of Fusion PCR amplicon for cloning into pFWKR to construct pFWKR-spy2136</b>		3842
NestNheISpyCIM1 (NheI)	TACCACTGAGCTAGCCTAAATAGCATGACAAGC	
NestXmaISpyCIM1 (XmaI)	GAACACAAGCCCGGGAAGTGCCTAAGT	
<b>SpyCIM1 Primase gene <i>spy2136</i> (PCR amplicon also used as Southern blot probe)</b>		950
SPy2136PrimaseFwd	TTGCTA AAGCAGAATACAGAAAGAGAGG	
Spy2136PrimaseRev	ATTACAATGGATTCCCTAGAAAGTATCG	
<b>SpyCIM1 Integrase (PCR amplicon also used as Southern blot probe)</b>		564
SpyCIM1intFwd	TAAAGCGTTCAATCCCTGCT	
SpyCIM1intRev	CTGCGGTACGTTCAATCATC	
<b>Across SpyCIM1 attP-L junction</b>		919
mutS-MutLattBFwd	TCGTGGCCTTTCTACAAC	
SpyCIM1intRev	CTGCGGTACGTTCAATCATC	
<b>Across SpyCIM1 attP-R junction</b>		429
SpyCIM1attRFwd	TACCCTGTAAAGACTGCC	
mutS-MutLattBRev	CAGGACTGCCAAAATCCCTA	
<b>Across SpyCIM1 attB in phage KO (PCR amplicon also used as Southern blot probe). From Scott <i>et al.</i> [13]<sup>a</sup></b>		461
mutS-MutLattBFwd	TCGCTGGCCTTTCTACAAC	
mutS-MutLattBRev	CAGGACTGCCAAAATCCCTA	

<sup>a</sup> Abbreviations used: Kan<sup>r</sup>, kanamycin-resistant; Sm<sup>r</sup>, streptomycin-sensitive; MCS, multiple cloning site.

<sup>b</sup> All plasmids and primers were derived from this study unless otherwise stated:

<sup>c</sup> All sequences are 5' to 3'. Restriction sites engineered into each primer are denoted in bold and the corresponding restriction enzymes are indicated in parentheses. Italicized text indicates overlapping primer sequences used for PCR based fusion and assembly of the Janus Cassette between the adjacent regions of phage genes targeted for allelic replacement.