

Table S5. Primers used in this study.

Name and purpose	Sequence (5' - 3') (with underlined feature)	Purpose/target	Feature
Production of pSinS-based vectors for gene insertional inactivation			
oII1538F	ccc <u>GGATCC</u> ATGCACGACAGGATTTGGGG	internal fragment of RS01780 gene (<i>pmi</i>)	<i>Bam</i> HI
oII1538R	ccc <u>GGATCC</u> TCTCCTTCATGTTACAGTCC	internal fragment of RS01780 gene (<i>pmi</i>)	<i>Bam</i> HI
M13F	GTTTTCCCAGTCACGACGTTGTA	DNA sequencing of plasmid insert	
M13R	CAGGAAACAGCTATGACC	DNA sequencing of plasmid insert	
oII1538V1	TACGGTTATATCACAACCACTCC	RS01780 mutant genotyping	
oII1538V2	AATAAAATCAATCATCGTAGTCACC	RS01780 mutant genotyping	
oII0569F	ccc <u>GGATCC</u> CAGATTTTTAGAAAACCGAACAGC	internal fragment of RS06460 gene (<i>sagH</i>)	<i>Bam</i> HI
oII0569R	ccc <u>GGATCC</u> AAAACAGCCGTTAAGGCTCC	internal fragment of RS06460 gene (<i>sagH</i>)	<i>Bam</i> HI
oII0569V1	CCGGTAAAATTACTGTTTTAGG	RS06460 mutant genotyping	
oII0569V2	CGTCACCTGCTTCTCATAACC	RS06460 mutant genotyping	
oII1333F	ccc <u>GGATCC</u> GGCTCTACTCAACCATTACC	internal fragment of RS02780 gene (<i>yvqE</i>)	<i>Bam</i> HI
oII1333R	ccc <u>GGATCC</u> CTTTTGCATGTTAGCAGTCAGG	internal fragment of RS02780 gene (<i>yvqE</i>)	<i>Bam</i> HI
oII1333V1	AACTACTCGTCATATAAAGATGC	RS02780 mutant genotyping	
oII1333V2	AAATCAACCCCTTCACGTCC	RS02780 mutant genotyping	
oII1784F	ccc <u>GGATCC</u> CGTCTTCTAACTGCTATTGGTGG	internal fragment of RS08695 gene (<i>ptsG</i>)	<i>Bam</i> HI
oII1784R	ccc <u>GGATCC</u> GTGAAAATCTCAGCCAACATGG	internal fragment of RS08695 gene (<i>ptsG</i>)	<i>Bam</i> HI
oII1784V1	ATCGGTTATTTGGTCAATGGTAACC	RS08695 mutant genotyping	
oII1784V2	ATATTTGAAAGAGCAATCATTGG	RS08695 mutant genotyping	
oII0955F	ccc <u>GGATCC</u> ATTCTTTTTGTTAAGTCTTTTGGC	internal fragment of RS04625 gene (<i>pstS</i>)	<i>Bam</i> HI
oII0955R	ccc <u>GGATCC</u> ACTGCAAGTCTTGCAACAGC	internal fragment of RS04625 gene (<i>pstS</i>)	<i>Bam</i> HI
oII0955V1	AAAATGAAGATGTTGTGCAGTGG	RS04625 mutant genotyping	
oII0955V2	AGAGCACCAAGATAAGGAATGC	RS04625 mutant genotyping	
oII0693F	ccc <u>GGATCC</u> CTAAAGGAAAGTCATTGTTATGC	internal fragment of RS05865 gene (<i>vfr</i>)	<i>Bam</i> HI
oII0693R	ccc <u>GGATCC</u> GAACTAAAACCTCAAGTCAACG	internal fragment of RS05865 gene (<i>vfr</i>)	<i>Bam</i> HI
oII0693V1	ACAAGTCTTAAATACCCATAACG	RS05865 mutant genotyping	
oII0693V2	TAGTATCATTAAATTTAATCATCTGC	RS05865 mutant genotyping	
oII1479F	ccc <u>GGATCC</u> GCCATGGTAAATTTGCTGAAGG	internal fragment of RS02065 gene (<i>manL</i>)	<i>Bam</i> HI
oII1479R	ccc <u>GGATCC</u> GTTCAGTGTAAAGCTTGAATAAGC	internal fragment of RS02065 gene (<i>manL</i>)	<i>Bam</i> HI
oII1479V1	TTAGAGATTACCAAAAATCACTCG	RS02065 mutant genotyping	
oII1479V2	AACGACCAAAATTCGAGAAATAATCG	RS02065 mutant genotyping	

Table S5. Primers used in this study (continued).

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Production of pSinS-based vectors for gene insertional inactivation			
oII1474F	ccc <u>GGATCC</u> CCTATTGTGTTAAACAACCTGTCTTGG	internal fragment of RS02090 gene (<i>cpsA</i>)	<i>Bam</i> HI
oII1474R	ccc <u>GGATCC</u> GATAACGTGGTTAAGGTATCTCG	internal fragment of RS02090 gene (<i>cpsA</i>)	<i>Bam</i> HI
oII1474V	TTTACTGTCTGAGACTTAGTTGACC	RS02090 mutant genotyping	
oII1073F	ccc <u>GGATCC</u> CAGTTTGCTCAGACACAGGC	internal fragment of RS04065 gene (<i>dltA</i>)	<i>Bam</i> HI
oII1073R	ccc <u>GGATCC</u> GTAAGAGGGAATTCCTCAATAGC	internal fragment of RS04065 gene (<i>dltA</i>)	<i>Bam</i> HI
oII1073V	AAGATAACTCCTTACCATCTTC	RS04065 mutant genotyping	
oII0543F	ccc <u>GGATCC</u> GCTTGCCAACCTTACTCAGGC	internal fragment of RS06590 gene (<i>acdA</i>)	<i>Bam</i> HI
oII0543R	ccc <u>GGATCC</u> CATGTCCAGCTCCTGCTACC	internal fragment of RS06590 gene (<i>acdA</i>)	<i>Bam</i> HI
oII0543V	GACGATCTTTGACTTCTTTGTC	RS06590 mutant genotyping	
oII0077F	ccc <u>GGATCC</u> GTAATACTATTTTATATAAAAGC	internal fragment of RS00535 gene (<i>acdR</i>)	<i>Bam</i> HI
oII0077R	ccc <u>GGATCC</u> AAGTCACCCTAGCATCAACC	internal fragment of RS00535 gene (<i>acdR</i>)	<i>Bam</i> HI
oII0077V	GGGTAATCATCAGAACGGATTTCC	RS00535 mutant genotyping	
oII1318F	ccc <u>GGATCC</u> GTAAGTGCAATTCGCTTCC	internal fragment of RS02880 gene (<i>rocA</i>)	<i>Bam</i> HI
oII1318R	ccc <u>GGATCC</u> GTAATTATGTTTAAACAACCTGAATAAG	internal fragment of RS02880 gene (<i>rocA</i>)	<i>Bam</i> HI
oII1318V	CTATAGCATTATCTAACAATAATGAC	RS02880 mutant genotyping	
oII0959F	ccc <u>GGATCC</u> GTCGTAAGCAAGGGCATGG	internal fragment of RS04605 gene (<i>spxA</i>)	<i>Bam</i> HI
oII0959R	ccc <u>GGATCC</u> TCCATGATAATAGGGCGACG	internal fragment of RS04605 gene (<i>spxA</i>)	<i>Bam</i> HI
oII0959V	ATCTGCCATCACATCATAAATAAGC	RS04605 mutant genotyping	
oII1724F	5' -ccc <u>GGATCC</u> GGTTACCTCTATTTTGTAGTGC - 3'	internal fragment of RS08425 gene (<i>ihk</i>)	<i>Bam</i> HI
oII1724R	5' -ccc <u>GGATCC</u> CACATTAGAATAAGAGTTGTCAAAGG - 3'	internal fragment of RS08425 gene (<i>ihk</i>)	<i>Bam</i> HI
oII1724V	5' -ACATGCTAAGGATACGCCGG - 3'	RS08425 mutant genotyping	
Production of <i>Krmit</i> insertion tags			
Adapter501A	TTCCCTACACGACGCTCTTCCGATCTTATAGCCTNN	<i>Mme</i> I Tnseq adapter	Adapter501
Adapter501B	<u>AGGCTATA</u> AAGATCGGAAGAGCGTCGTGTAGGGAAAGAG	<i>Mme</i> I Tnseq adapter	Adapter501
Adapter502A	TTCCCTACACGACGCTCTTCCGATCTATAGAGGCNN	<i>Mme</i> I Tnseq adapter	Adapter502
Adapter502B	<u>GCCTCTATA</u> GATCGGAAGAGCGTCGTGTAGGGAAAGAG	<i>Mme</i> I Tnseq adapter	Adapter502
Adapter503A	TTCCCTACACGACGCTCTTCCGATCTCCTATCCTNN	<i>Mme</i> I Tnseq adapter	Adapter503
Adapter503B	<u>AGGATAGG</u> GATCGGAAGAGCGTCGTGTAGGGAAAGAG	<i>Mme</i> I Tnseq adapter	Adapter503
Adapter504A	TTCCCTACACGACGCTCTTCCGATCTGGCTCTGANN	<i>Mme</i> I Tnseq adapter	Adapter504
Adapter504B	<u>TCAGAGCC</u> CAGATCGGAAGAGCGTCGTGTAGGGAAAGAG	<i>Mme</i> I Tnseq adapter	Adapter504

Table S5. Primers used in this study (continued).

Name and purpose	Sequence (5' - 3') (with underlined feature)	Role	Feature
Production of <i>Krmit</i> insertion tags			
Adapter505A	TTCCCTACACGACGCTCTTCCGATCT <u>AGGCCGAA</u> GNN	<i>Mmel</i> Tnseq adapter	Adapter505
Adapter505B	<u>CTTCGCCTAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter505
Adapter506A	TTCCCTACACGACGCTCTTCCGATCT <u>TAATCTTANN</u>	<i>Mmel</i> Tnseq adapter	Adapter506
Adapter506B	<u>TAAGATTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter506
Adapter507A	TTCCCTACACGACGCTCTTCCGATCT <u>CAGGACGTNN</u>	<i>Mmel</i> Tnseq adapter	Adapter507
Adapter507B	<u>ACGTCTTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter507
Adapter508A	TTCCCTACACGACGCTCTTCCGATCT <u>GTACTGACNN</u>	<i>Mmel</i> Tnseq adapter	Adapter508
Adapter508B	<u>GTCAGTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter508
Adapter701A	TTCCCTACACGACGCTCTTCCGATCT <u>ATTACTCGNN</u>	<i>Mmel</i> Tnseq adapter	Adapter701
Adapter701B	<u>CGAGTAATAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter701
Adapter702A	TTCCCTACACGACGCTCTTCCGATCT <u>TCCGGAGANN</u>	<i>Mmel</i> Tnseq adapter	Adapter702
Adapter702B	<u>TCTCCGGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter702
Adapter703A	TTCCCTACACGACGCTCTTCCGATCT <u>CGCTCATTNN</u>	<i>Mmel</i> Tnseq adapter	Adapter703
Adapter703B	<u>AATGAGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter703
Adapter704A	TTCCCTACACGACGCTCTTCCGATCT <u>GAGATTCCNN</u>	<i>Mmel</i> Tnseq adapter	Adapter704
Adapter704B	<u>GGAATCTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAG</u>	<i>Mmel</i> Tnseq adapter	Adapter704
oKrmit-Tnseq2	CAAGCAGAAGACGGCATAACGAAAGCGCCTACGAGGAATTTGTATCG	PCR of <i>Krmit</i> insertion tags	
oAdapterPCR	AATGATACGGCGACCACCGAGATCACACTCTTCCCTACACGACGCTCTTCC	PCR of <i>Krmit</i> insertion tags	
Production of pCRS-based vectors for allelic exchange in the <i>scfAB</i> locus			
oKmF	ATGGCTAAAATGAGAATATCACC	promoterless <i>aphA3</i> ORF	
oKmR	CTAAAACAATTCATCCAGTAAAATA	promoterless <i>aphA3</i> ORF	
oAX0478.1	ccc <u>GGATCCC</u> GTAAGTACTGATCTGTCAGTTTTTCC	DNA sequence upstream of <i>scfA</i>	<i>Bam</i> HI
oAX0478.2	GGTGATATTCTCATT TTTAGCCATTTCACCATATCCTTTCTAGATGATAAAAAG	DNA sequence upstream of <i>scfA</i>	<i>aphA3</i> -tail
oAX0478.3	TATTTTACTGGATGAATTGTTTTAG ATATATGTCCAACTCGTGTGTTG	DNA sequence downstream of <i>scfA</i>	<i>aphA3</i> -tail
oAX0478.4	ccc <u>GGATCC</u> ATTTTTCAGTGGTAATGGTGACC	DNA sequence downstream of <i>scfA</i>	<i>Bam</i> HI
oAX0478.V1	TAATGGGAACAAGCCTGCTTCACG	<i>PscfA-aphA3</i> junction genotyping	
oAX0478.V2	GTGAATAATGCCGAAGCGG	<i>PscfA-aphA3</i> junction genotyping	
oKmV1	TAGCAGGAGACATTCTTCC	<i>PscfA-aphA3</i> junction genotyping	
oKmV2	TCGAGCTATTTTTTGACTTACTGG	<i>scfA</i> downstream junction genotyping	
oAX0477.1	ccc <u>GGATCC</u> GGTTCAAAAATACCTACCTAAGC	DNA sequence upstream of <i>scfB</i>	<i>Bam</i> HI

Table S5. Primers used in this study (continued).

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Production of pCRS-based vectors for allelic exchange in the <i>scfAB</i> locus			
oAX0477.2	GGTGATATTCTCATT <u>TTTAGCC</u> ATATAGCCAGCTAAAAATAAAAAGCG	DNA sequence upstream of <i>scfB</i>	<i>aphA3</i> -tail
oAX0477.3	TATTTTACTGGATGAATTG <u>TTTTAG</u> AGACTAATAAAAAAGCACTTGTTTTTTATC	DNA sequence downstream of <i>scfB</i>	<i>aphA3</i> -tail
oAX0477.4	ccc <u>GGATCC</u> GATATACTTGCTGCGCATGC	DNA sequence downstream of <i>scfB</i>	<i>Bam</i> HI
oAX0477.V1	CAATGGTTTGCGATTTTTATGTCC	<i>scfB</i> - <i>aphA3</i> junction genotyping	
oAX0477.V2	CTAAAAATGGATATTGATTTCACTTCAAAAGGC	<i>scfB</i> downstream junction genotyping	
Verification of the expression of <i>scfA</i> and <i>scfB</i> in the Δ<i>scfA</i> and Δ<i>scfB</i> mutants			
scfA M1T1 RT L	CGCATTCTGTTTGGAAACCT		
scfA M1T1 RT R	TTTTTCCAAAAAGCGGTTG		
scfB M1T1 RT L	CAAGGCTGACGAGTCCTTT		
scfB M1T1 RT R	CAGAAACCGTGGTGAATC		