Table S1. *Escherichia coli* K12 Strains and Plasmids Used in This Study*

Plasmid/ Strain	Relevant Genotype	Reference or Source
pKD3	Source of FRT*cat*FRT	(Datsenko and Wanner, 2000)
pKD4	Source of FRTKanFRT	(Datsenko and Wanner, 2000)
pKD46	*ori101 repA101ts* P_{BAD}-*gam-bet-exo* Amp^R	(Datsenko and Wanner, 2000)
pBBR1MCS2	Broad-host-range vector; Kan^R	(Kovach et al., 1995)
pCP20	Yeast Flp recombinase on a temperature-sensitive replicon λp_r-*FLP* , λc*Its857*, RepTS, Amp^R, Cam^R	(Cherepanov and Wackernagel, 1995)
BW25113	[pKD46]	(Datsenko and Wanner, 2000)
FC36	Δ(*lac-proB*)$_{XIII}$ *ara thi* Rif^R	(Cairns and Foster, 1991)
FC40	Δ(*lac-proB*)$_{XIII}$ *ara thi* Rif^R [F' *lacI33*Ω*lacZ proAB*$^+$]	(Cairns and Foster, 1991)
JW2787	Δ*recD*::FRTKanFRT	(Baba et al., 2006)
SMR3525	FC40Δ*umuCD*::*cat*	(McKenzie et al., 2000)
SMR4336	FC40 [F' *mhpC34*::Tn*10*d*tetA+1*]	(Bull et al., 2001)
SMR4562	Independent construction of FC40	(McKenzie et al., 2000)
SMR5822	BW25113 [pKD46]	(McKenzie et al., 2003)
SMR5833	SMR4562 [pKD46]	(McKenzie et al., 2003)
SMR5875	Δ*dinB50*::FRTKanFRT	(McKenzie, 2002)
SMR6233	MG1655 [pKD46]	MG1655 x pKD46
SMR6272	SMR4562 Δ*araBAD*567	(Ponder et al., 2005)
SMR7255	FC36 *leu*::Tn*10 ara*+	Lab collection
SMR7258	FC36 Δ*attλ*::P_{BAD}I-*Sce*I *leu*::Tn*10*	(Ponder et al., 2005)
SMR10769	FC40 [F' *mhpC34*::Tn*10*d*tetA+1*] [pKD46]	SMR4336 x pKD46
SMR10770	FC40 [F' *mhpC34*::Tn*10*d*tetA+1*FRT*cat*FRT]	SMR10769 x Short homology from pKD3 using primers P1 and P2
SMR10771	SMR4562 KanISceIsiteA'	SMR5833 x Short homology from pBBR1MCS2 using primer P57 and P58
SMR10772	FC36 Δ*araBAD*567	SMR7255 x P1 (SMR6272)
SMR10774	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I	SMR7258 x P1 (SMR6272)
SMR10777	SMR4562 3ChiKanISceIsiteA	SMR5833 x Short homology from pBBR1MCS2 using primers P3 and P4
SMR10782	SMR4562 *tet3*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P9 and P10
SMR10785	FC36 Δ*araBAD*567 *tet3*FRT*cat*FRT	SMR10772 x P1 (SMR10782)
SMR10786	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet3*FRT*cat*FRT	SMR10774 x P1 (SMR10782)
SMR10787	FC36 Δ*araBAD*567 *tet3*FRT*cat*FRT 3ChiKanISceIsiteA	SMR10785 x P1

Plasmid/ Strain	Relevant Genotype	Reference or Source
		(SMR10777)
SMR10788	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet3*FRT*cat*FRT 3ChiKanISceIsiteA	SMR10786 x P1 (SMR10777)
SMR10789	SMR4562 3ChiKanISceIsiteA [pKD46]	SMR10777 x pKD46
SMR10796	SMR4562 *tet2*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P5 and P6
SMR10797	FC36 Δ*araBAD*567 *tet2*FRT*cat*FRT	SMR10772 x P1 (SMR10796)
SMR10798	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT*cat*FRT	SMR10774 x P1 (SMR10796)
SMR10799	FC36 Δ*araBAD*567 *tet2*FRT*cat*FRT 3ChiKanISceIsiteA	SMR10797 x P1 (SMR10777)
SMR10800	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT*cat*FRT 3ChiKanISceIsiteA	SMR10798 x P1 (SMR10777)
SMR10804	SMR4562 *tet17*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P45 and P46
SMR10805	FC36 Δ*araBAD*567 *tet17*FRT*cat*FRT	SMR10772 x P1 (SMR10804)
SMR10806	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet17*FRT*cat*FRT	SMR10774 x P1 (SMR10804)
SMR10807	FC36 Δ*araBAD*567 *tet2*FRT	SMR10797 x pCP20
SMR10808	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT	SMR10798 x pCP20
SMR10809	FC36 Δ*araBAD*567 *tet17*FRT*cat*FRT 3ChiKanISceIsiteA	SMR10805 x P1 (SMR10777)
SMR10810	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet17*FRT*cat*FRT 3ChiKanISceIsiteA	SMR10806 x P1 (SMR10777)
SMR10815	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT Δ*recD*::FRTKanFRT	SMR10808 x P1 JW2787
SMR10828	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT Δ*recD*::FRT	SMR10815 x pCP20
SMR10850	FC36 Δ*araBAD*567 *tet2*FRT 3ChiKanISceIsiteA	SMR10799 x pCP20
SMR10858	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT Δ*recD*::FRT 3ChiKanISceIsiteA	SMR10828 x P1 (SMR10777)
SMR10865	FC36 Δ*araBAD*567 *tet2*FRT 3ChiKanISceIsiteA	SMR10807 x P1 (SMR10850)
SMR10866	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT 3ChiKanISceIsiteA	SMR10808 x P1 (SMR10850)
SMR10868	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT 3ChiKanISceIsiteA Δ*dinB50*::FRT	(Shee et al., 2011)
SMR10896	SMR4562 3ChiKanISceIsiteA *tet1*FRT*cat*FRT	SMR10789 x Short homology from SMR10770 using primers P7 and P8
SMR10900	FC36 Δ*araBAD*567 3ChiKanISceIsiteA *tet1*FRT*cat*FRT	SMR10772 x P1 (SMR10896)
SMR10901	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I 3ChiKanISceIsiteA *tet1*FRT*cat*FRT	SMR10774 x P1 (SMR10896)
SMR11694	SMR4562 *tet9*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P19 and P20
SMR11722	FC36 Δ*araBAD*567 *tet9*FRT*cat*FRT	SMR10772 x P1 (SMR11694)
SMR11723	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet9*FRT*cat*FRT	SMR10774 x P1

Plasmid/ Strain	Relevant Genotype	Reference or Source
		(SMR11694)
SMR11724	FC36 Δ*araBAD*567 *tet9*FRT*cat*FRT 3ChiKanISceIsiteA	SMR11722 x P1 (SMR10777)
SMR11725	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet9*FRT*cat*FRT 3ChiKanISceIsiteA	SMR11723 x P1 (SMR10777)
SMR11763	SMR4562 3ChiKanISceIsiteB	SMR5833 x Short homology from pBBR1MCS2 using primers P21 and P22
SMR11764	FC36 Δ*araBAD*567 *tet9*FRT*cat*FRT 3ChiKanISceIsiteB	SMR11722 x P1 (SMR11763)
SMR11765	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet9*FRT*cat*FRT 3ChiKanISceIsiteB	SMR11723 x P1 (SMR11763)
SMR11768	SMR4562 3ChiKanISceIsiteC	SMR5833 x Short homology from pBBR1MCS2 using primers P25 and P26
SMR11769	SMR4562 *tet13*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P27 and P28
SMR11773	FC36 Δ*araBAD*567 *tet13*FRT*cat*FRT	SMR10772 x P1 (SMR11769)
SMR11774	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT	SMR10774 x P1 (SMR11769)
SMR11775	FC36 Δ*araBAD*567 *tet13*FRT*cat*FRT 3ChiKanISceIsiteA	SMR11773 x P1 (SMR10777)
SMR11776	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT 3ChiKanISceIsiteA	SMR11774 x P1 (SMR10777)
SMR11777	FC36 Δ*araBAD*567 *tet13*FRT*cat*FRT 3ChiKanISceIsiteC	SMR11773 x P1 (SMR11768)
SMR11778	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT 3ChiKanISceIsiteC	SMR11774 x P1 (SMR11768)
SMR11784	SMR4562 *tet10*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P31 and P32
SMR11785	SMR4562 *tet4*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P11 and P12
SMR11787	FC36 Δ*araBAD*567 *tet10*FRT*cat*FRT	SMR10772 x P1 (SMR11784)
SMR11788	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet10*FRT*cat*FRT	SMR10774 x P1 (SMR11784)
SMR11789	FC36 Δ*araBAD*567 3ChiKanISceIsiteA *tet10*FRT*cat*FRT	SMR11787 x P1 (SMR10777)
SMR11790	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I 3ChiKanISceIsiteA *tet10*FRT*cat*FRT	SMR11788 x P1 (SMR10777)
SMR11801	FC36 Δ*araBAD*567 *tet4*FRT*cat*FRT	SMR10772 x P1 (SMR11785)
SMR11802	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT*cat*FRT	SMR10774 x P1 (SMR11785)
SMR11841	FC36 Δ*araBAD*567 *tet5*FRT*cat*FRT	SMR10772 x P1

Plasmid/ Strain	Relevant Genotype	Reference or Source
		(SMR12439)
SMR12437	FC36 Δ*araBAD*567 *tet4*FRT*cat*FRT 3ChiKanISceIsiteA	SMR11801 x P1 (SMR10777)
SMR12438	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT*cat*FRT 3ChiKanISceIsiteA	SMR11802 x P1 (SMR10777)
SMR12439	SMR4562 *tet5*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P13 and P14
SMR12440	SMR4562 *tet6*FRT*cat*FRT	SMR5833 x Short homology from SMR10770 using primers P15 and P16
SMR12442	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet5*FRT*cat*FRT	SMR10774 x P1 (SMR12439)
SMR12443	FC36 Δ*araBAD*567 *tet6*FRT*cat*FRT	SMR10772 x P1 (SMR12440)
SMR12444	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT	SMR10774 x P1 (SMR12440)
SMR12445	FC36 Δ*araBAD*567 *tet5*FRT*cat*FRT 3ChiKanISceIsiteA	SMR11841 x P1 (SMR10777)
SMR12446	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet5*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12442 x P1 (SMR10777)
SMR12447	FC36 Δ*araBAD*567 *tet6*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12443 x P1 (SMR10777)
SMR12448	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12444 x P1 (SMR10777)
SMR12449	SMR5822 *tet12*FRT*cat*FRT	SMR5822 x Short homology from SMR10770 using primers P23 and P24
SMR12450	SMR5822 *tet14*FRT*cat*FRT	SMR5822 x Short homology from SMR10770 using primers P37 and P38
SMR12453	SMR5822 *tet7*FRT*cat*FRT	SMR5822 x Short homology from SMR10770 using primers P17 and P18
SMR12454	FC36 Δ*araBAD*567 *tet7*FRT*cat*FRT	SMR10772 x P1 (SMR12453)
SMR12455	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT	SMR10774 x P1 (SMR12453)
SMR12456	FC36 Δ*araBAD*567 *tet7*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12454 x P1 (SMR10777)
SMR12457	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12455 x P1 (SMR10777)
SMR12459	SMR5822 *tet15*FRT*cat*FRT	SMR5822 x Short homology from SMR10770 using primers P39 and P40
SMR12462	FC36 Δ*araBAD*567 *tet12*FRT*cat*FRT	SMR10772 x P1 (SMR12449)

Plasmid/ Strain	Relevant Genotype	Reference or Source
SMR12463	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet12*FRT*cat*FRT	SMR10774 x P1 (SMR12449)
SMR12468	FC36 Δ*araBAD*567 *tet15*FRT*cat*FRT	SMR10772 x P1 (SMR12459)
SMR12469	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet15*FRT*cat*FRT	SMR10774 x P1 (SMR12459)
SMR12470	FC36 Δ*araBAD*567 *tet12*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12462 x P1 (SMR11763)
SMR12471	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet12*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12463 x P1 (SMR11763)
SMR12476	FC36 Δ*araBAD*567 *tet15*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12468 x P1 (SMR11763)
SMR12477	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet15*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12469 x P1 (SMR11763)
SMR12478	FC36 Δ*araBAD*567 *tet14*FRT*cat*FRT	SMR10772 x P1 (SMR12450)
SMR12479	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet14*FRT*cat*FRT	SMR10774 x P1 (SMR12450)
SMR12480	SMR5822 *tet2'*FRT*cat*FRT	SMR5822 x Short homology from SMR10770 using primers P43 and P44
SMR12481	FC36 Δ*araBAD*567 *tet14*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12478 x P1 (SMR11763)
SMR12482	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet14*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12479 x P1 (SMR11763)
SMR12483	FC36 Δ*araBAD*567 *tet2'*FRT*cat*FRT	SMR10772 x P1 (SMR12480)
SMR12484	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2'*FRT*cat*FRT	SMR10774 x P1 (SMR12480)
SMR12485	FC36 Δ*araBAD*567 *tet2'*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12483 x P1 (SMR10777)
SMR12486	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2'*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12484 x P1 (SMR10777)
SMR12487	FC36 Δ*araBAD*567 *tet2'*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12485 x pCP20
SMR12488	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2'*FRT*cat*FRT 3ChiKanISceIsiteA	SMR12486 x pCP20
SMR12489	FC36 Δ*araBAD*567 *tet2*FRT*cat*FRT KanISceIsiteA'	SMR10797 x P1 (SMR10771)
SMR12494	SMR5822 *tet16*FRT*cat*FRT	SMR5822 x Short homology from SMR10770 using primers P41 and P42
SMR12497	FC36 Δ*araBAD*567 3ChiKanISceIsiteA	SMR10772 x P1 (SMR10777)
SMR12498	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I 3ChiKanISceIsiteA	SMR10774 x P1 (SMR10777)
SMR12505	SMR5822 *tet11*FRT*cat*FRT	SMR5822 x Short homology from SMR10770 using primers P35 and P36
SMR12725	SMR5822 3ChiKanISceIsiteD	SMR5833 x Short homology from pBBR1MCS2 using primers P33 and P34
SMR12726	SMR5833 *tet8*FRT*cat*FRT	SMR5833 x Short

Plasmid/ Strain	Relevant Genotype	Reference or Source
		homology from SMR10770 using primer P29 and P30
SMR12728	SMR4562 KanISceIsiteB'	SMR5833 x Short homology from pBBR1MCS2 using primer P59 and P60
SMR12729	FC36 Δ*araBAD*567 *tet10*FRT*cat*FRT 3ChiKanISceIsiteD	SMR11787 x P1 (SMR12725)
SMR12730	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet10*FRT*cat*FRT 3ChiKanISceIsiteD	SMR11788 x P1 (SMR12725)
SMR12731	FC36 Δ*araBAD*567 3ChiKanISceIsiteA *tet8*FRT*cat*FRT	SMR12497 x P1 (SMR12726)
SMR12732	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I 3ChiKanISceIsiteA *tet8*FRT*cat*FRT	SMR12498 x P1 (SMR12726)
SMR12735	FC36 Δ*araBAD*567 *tet14*FRT*cat*FRT KanISceIsiteB'	SMR12478 x P1 (SMR12728)
SMR12736	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet14*FRT*cat*FRT KanISceIsiteB'	SMR12479 x P1 (SMR12728)
SMR12737	FC36 Δ*araBAD*567 *tet16*FRT*cat*FRT	SMR10772 x P1 (SMR12494)
SMR12738	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet16*FRT*cat*FRT	SMR10774 x P1 (SMR12494)
SMR12739	FC36 Δ*araBAD*567 *tet11*FRT*cat*FRT	SMR10772 x P1 (SMR12505)
SMR12740	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet11*FRT*cat*FRT	SMR10774 x P1 (SMR12505)
SMR12744	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT*cat*FRT Δ*dinB50*::FRTKanFRT	SMR11802 x P1 (SMR5875)
SMR12747	FC36 Δ*araBAD*567 *tet16*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12737 x P1 (SMR11763)
SMR12748	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet16*FRT*cat*FRT 3ChiKanISceIsiteB	SMR12738 x P1 (SMR11763)
SMR12749	FC36 Δ*araBAD*567 3ChiKanISceIsiteB *tet11*FRT*cat*FRT	SMR12739 x P1 (SMR11763)
SMR12750	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I 3ChiKanISceIsiteB *tet11*FRT*cat*FRT	SMR12740 x P1 (SMR11763)
SMR12753	FC36 Δ*araBAD*567 *tet11*FRT*cat*FRT KanISceIsiteB'	SMR12739 x P1 (SMR12728)
SMR12754	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet11*FRT*cat*FRT KanISceIsiteB'	SMR12740 x P1 (SMR12728)
SMR12758	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT*cat*FRT Δ*dinB50*::FRT	SMR12744 x pCP20
SMR12764	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT*cat*FRT Δ*dinB50*::FRT 3ChiKanISceIsiteA	SMR12758 x P1 (SMR10777)
SMR12794	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT Δ*dinB50*::FRTKanFRT	SMR12444 x P1 (SMR5875)
SMR12796	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT Δ*dinB50*::FRTKanFRT	SMR12455 x P1 (SMR5875)
SMR12798	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT Δ*dinB50*::FRT	SMR12794 x pCP20
SMR12800	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT Δ*dinB50*::FRT	SMR12796 x pCP20
SMR12801	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*polB*::FRT*cat*FRT	Shee et al. 2011
SMR12810	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT 3ChiKanISceIsiteA Δ*dinB50*::FRT Δ*polB*::FRT*cat*FRT	SMR10868 x P1 (SMR12801)
SMR12814	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT*cat*FRT Δ*dinB50*::FRT 3ChiKanISceIsiteA Δ*polB*::FRT*cat*FRT	SMR12764 x P1 (SMR12801)
SMR13124	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT Δ*dinB50*::FRT 3ChiKanISceIsiteA	SMR12798 x P1 (SMR10777)
SMR13126	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT Δ*dinB50*::FRT 3ChiKanISceIsiteA	SMR12800 x P1 (SMR10777)

Plasmid/ Strain	Relevant Genotype	Reference or Source
SMR13128	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT Δ*dinB50*::FRT Δ*polB*::FRT*cat*FRT	SMR12798 x P1 (SMR12801)
SMR13130	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT Δ*dinB50*::FRT Δ*polB*::FRT*cat*FRT	SMR12800 x P1 (SMR12801)
SMR13153	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT Δ*dinB50*::FRT Δ*polB*::FRT*cat*FRT 3ChiKanISceIsiteA	SMR13128 x P1 (SMR10777)
SMR13155	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT Δ*dinB50*::FRT Δ*polB*::FRT*cat*FRT 3ChiKanISceIsiteA	SMR13130 x P1 (SMR10777)
SMR14582	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT 3ChiKanISceIsiteA	SMR12438 x pCP20
SMR14586	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT 3ChiKanISceIsiteA	SMR12448 x pCP20
SMR14588	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT 3ChiKanISceIsiteA	SMR12457 x pCP20
SMR14590	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT 3ChiKanISceIsiteA Δ*dinB50*::FRT Δ*umuCD*::*cat*	SMR10868 x P1 (SMR3525)
SMR14594	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT*cat*FRT Δ*dinB50*::FRT 3ChiKanISceIsiteA Δ*umuCD*::*cat*	SMR12764 x P1 SMR3525
SMR14598	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT*cat*FRT Δ*dinB50*::FRT 3ChiKanISceIsiteA Δ*umuCD*::*cat*	SMR13124 x P1 (SMR3525)
SMR14600	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT*cat*FRT Δ*dinB50*::FRT 3ChiKanISceIsiteA Δ*umuCD*::*cat*	SMR13126 x P1 (SMR3525)
SMR14616	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT 3ChiKanISceIsiteA	SMR10828 x P1 (SMR10777)
SMR14618	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tetRtetA+1*FRT*cat*FRT(*tet1)* Δ*recD*::FRT 3ChiKanISceIsiteA	SMR14616 x P1 (SMR10896)
SMR14620	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT 3ChiKanISceIsiteA *tet3*FRT*cat*FRT	SMR14616 x P1 (SMR10782)
SMR14622	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT 3ChiKanISceIsiteA *tet4*FRT*cat*FRT	SMR14616 x P1 (SMR11785)
SMR14624	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT 3ChiKanISceIsiteA *tet5*FRT*cat*FRT	SMR14616 x P1 (SMR12439)
SMR14626	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT 3ChiKanISceIsiteA *tet6*FRT*cat*FRT	SMR14616 x P1 (SMR12440)
SMR14628	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT ChiKanISceIsiteA *tet7*FRT*cat*FRT	SMR14616 x P1 (SMR12453)
SMR14630	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT ChiKanISceIsiteA *tet8*FRT*cat*FRT	SMR14616 x P1 (SMR12726)
SMR14632	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT 3ChiKanISceIsiteA *tet9*FRT*cat*FRT	SMR14616 x P1 (SMR11694)
SMR14684	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet2*FRT 3ChiKanISceIsiteA Δ*umuCD*::*cat*	SMR10866 x P1 (SMR3525)
SMR14586	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT 3ChiKanISceIsiteA	SMR12448 x pCP20
SMR14588	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT 3ChiKanISceIsiteA	SMR12457 x pCP20
SMR14688	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet4*FRT 3ChiKanISceIsiteA Δ*umuCD*::*cat*	SMR14582 x P1 (SMR3525)
SMR14692	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet6*FRT 3ChiKanISceIsiteA Δ*umuCD*::*cat*	SMR14586 x P1 (SMR3525)
SMR14694	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet7*FRT 3ChiKanISceIsiteA Δ*umuCD*::*cat*	SMR14588 x P1 (SMR3525)
SMR16374	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRTKanFRT	SMR 10774 x P1 JW2787
SMR16392	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT	SMR16374 x pCP20
SMR16673	MG1655 FRTKanFRTISceIsiteE	SMR6233x Short homology from pKD4 using primers P47-P48
SMR16674	MG1655 FRTKanFRTISceIsiteF	SMR6233x Short homology from pKD4 using primers P49-P50

Plasmid/ Strain	Relevant Genotype	Reference or Source
SMR16675	MG1655 FRTKanFRTISceIsiteG	SMR6233x Short homology from pKD4 using primers P50-P52
SMR16700	MG1655 FRTKanFRTISceIsiteH	SMR6233x Short homology from pKD4 using primers P53-P54
SMR16701	MG1655 FRTKanFRTISceIsiteI	SMR6233x Short homology from pKD4 using primers P55-P56
SMR16729	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT FRTKanFRTISceIsiteE	SMR11774 x P1 (SMR16673)
SMR16731	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT FRTKanFRTISceIsiteF	SMR11774 x P1 (SMR16674)
SMR16733	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT FRTKanFRTISceIsiteG	SMR11774 x P1 (SMR16675)
SMR16735	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT FRTKanFRTISceIsiteH	SMR11774 x P1 (SMR16700)
SMR16737	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I *tet13*FRT*cat*FRT FRTKanFRTISceIsiteI	SMR11774 x P1 (SMR16701)
SMR16739	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT *tet13*FRT*cat*FRT	SMR16392 x P1 (SMR11769)
SMR16746	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT *tet13*FRT*cat*FRT FRTKanFRTISceIsiteE	SMR16739 x P1 (SMR16673)
SMR16748	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT *tet13*FRT*cat*FRT FRTKanFRTISceIsiteF	SMR16739 x P1 (SMR16674)
SMR16750	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT *tet13*FRT*cat*FRT FRTKanFRTISceIsiteG	SMR16739 x P1 (SMR16675)
SMR16752	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT *tet13*FRT*cat*FRT FRTKanFRTISceIsiteH	SMR16739 x P1 (SMR16700)
SMR16754	FC36 Δ*araBAD*567 Δ*attλ*::P_{BAD}I-*Sce*I Δ*recD*::FRT *tet13*FRT*cat*FRT FRTKanFRTISceIsiteI	SMR16739 x P1 (SMR16701)

**E. coli* strains and plasmids were constructed using standard P1 transduction, transformation (Miller, 1992), lysogenization (Gumbiner-Russo et al., 2001), recombinant DNA (Sambrook and Russell, 2001) and short-homology recombineering methods (Datsenko and Wanner, 2000). Sequencing was performed by SeqWright DNA Technology Services (Houston, TX).

Table S2. Names and Locations of New I-Sites and *tet* Alleles

Short allele name used in text and figures	Allele description*	Minute position	Insertion/deletion site†
I-site A	*zie3920.5*::3ChiKanISceIsiteA	~84.2'	3920551 - 3920571
I-site A'	*zie3920.5'*::KanISceIsiteA'	~84.2'	3920551 - 3920571
I-site B	*zfd2513.5*::3ChiKanISceIsiteB	~53.99'	2513573 - 2513593
I-site B'	*zfd2513.5'*::KanISceIsiteB'	~53.99'	2513573 - 2513593
I-site C	*zie3936.8*::3ChiKanISceIsiteC	~84.55'	3936888 - 3936907
I-site D	*zdd1542.3*::3ChiKanISceIsiteD	~33.12'	1542348 - 1542374
I-site E	*zif3960.3*::FRTKanFRTISceIsiteE	~85.06'	3960326 - 3960337
I-site F	*zif3981.8*::FRTKanFRTISceIsiteF	~85.54'	3981825 - 3981831
I-site G	*zig4005.9*::FRTKanFRTISceIsiteG	~86.05'	4005988 - 4006012
I-site H	*zih4077.5*::FRTKanFRTISceIsiteH	~87.58'	4077589 - 4077599
I-site I	*zii4124.8*::FRTKanFRTISceIsiteI	~88.6'	4124854 - 4124874
tet1	*zie3920.3*::*tetRtetA+1*FRT*cat*FRT	~84.2'	3920355 - 3920375
tet2	*zie3913.1*::*tetRtetA+1*FRT	~84.06'	3913117 - 3913122
tet2'	*zie3913.1'*::*tetRtetA+1*FRT	~84.06'	3913117 - 3913122
tet2a	*zie3913.1*::*tetRtetA+1*FRT*cat*FRT	~84.06'	3913117 - 3913122
tet3	*zid3892.8*::*tetRtetA+1*FRT*cat*FRT	~83.6'	3892884 - 3892891
tet4	*zic3858.9*::*tetRtetA+1*FRT*cat*FRT	~82.88'	3858910 - 3858939
tet5	*zic3829.8*::*tetRtetA+1*FRT*cat*FRT	~82.25'	3829822 - 3829836
tet6	*zib3786.6*::*tetRtetA+1*FRT*cat*FRT	~81.3'	3786668 - 3786676
tet7	*zhi3661.4*::*tetRtetA+1*FRT*cat*FRT	~78.65'	3661454 - 3661477
tet8	*zhc3388.1*::*tetRtetA+1*FRT*cat*FRT	~72.78'	3388124 - 3388141
tet9	*zfd2509.2*::*tetRtetA+1*FRT*cat*FRT	~53.9'	2509244 - 2509256
tet10	*zdc1532.9*::*tetRtetA+1*FRT*cat*FRT	~32.92'	1532951 - 1532970
tet11	*zfd2502.5*::*tetRtetA+1*FRT*cat*FRT	~53.75'	2502502 - 2502528
tet12	*zfd2474.4*::*tetRtetA+1*FRT*cat*FRT	~53.15'	2474465 - 2474539
tet13	*zie3954.5*::*tetRtetA+1*FRT*cat*FRT	~84.94'	3954539 - 3954562
tet14	*zfe2523.9*::*tetRtetA+1*FRT*cat*FRT	~54.22'	2523923 - 2523949
tet15	*zfe2547.5*::*tetRtetA+1*FRT*cat*FRT	~54.72'	2547597 - 2547638
tet16	*zff2572.2*::*tetRtetA+1*FRT*cat*FRT	~55.25'	2572275 - 2572296
tet17	*zie3927.2*::*tetRtetA+1*FRT*cat*FRT	~84.35'	3927225 - 3927226

***** Insertion sites named according to nomenclature for insertions not within a particular gene (Chumley et al., 1979).

† I-*Sce*I cutsites (I-sites) and *tet* alleles were inserted, deleting base pairs between coordinates shown, using phage λ Red-mediated short-homology recombineering methods (Datsenko and Wanner, 2000) with donor template sequences and steps outlined in **Supplementary Table 1.**

Table S3. PCR Primers

Primer	Sequence (5' - 3')	Strain	Cassette	Allele	Deletion coordinates (Minute position)
P1	CCGTTTCCATTTAGGTGGGTAC GTTGGAGCCGCATTATTTTGTGT AGGCTGGAGCTGCTT	SMR10770	*tetA*		
P2	CTTCACGGTAACCAAGATGTCG AGTTAACCACCCTTTAGACATAT GAATATCCTCCTTAG				
P3	ACTGGTTACATTTAACGCCACGT TCACTCTTTTGCATCAACAAGAG CTGGTGGGCTGGTGGCTGGTG GGGATGAATGTCAGCTAC	SMR10777	I-site A	*zie3920.5*	3920551 - 3920571 (~84.2')
P4	CGATTTTTAGCAGACTGATATTT TCACTAATGACTTATTTTCTGCA TTACCCTGTTATCCCTACGAAAT CTCGTGATGGCAGG				
P5	GCCAGTCTGGAAACAGGCTGGC TTTTTTTTGCGCGTGTGCCGTTT CCATTTAGGTGGGTA	SMR10796	*tet*2	*zie3913.1*	3913117 - 3913122 (~84.06')
P6	GGTTTTATATCAGCAGGATCTAT GTGAACGCTATTCAGGCGCGGA ATAACATCATTTGGT				
P7	CTGTTTTTAGACTGAAATATCAT AAACTTGCAAAGGCATCCCGTTT CCATTTAGGTGGGT	SMR10896	*tet*1	*zie3920.3*	3920355 - 3920375 (~84.2')
P8	GCGTGCTTCAGATCACATATTG CGCATGTTCGCGCACAGCCGCG GAATAACATCATTTGG				
P9	GAAGATTGTCTTTATCGACGCTC ACTAAAATCGATAGCGCCCGTTT CCATTTAGGTGGGT	SMR10782	*tet*3	*zid3892.8*	3892884 - 3892891 (~83.6')
P10	GATCTAAATAAAAAACCCGCCA GCAATCATGCATGGCGGGCGC GGAATAACATCATTTGG				
P11	GGTTAATGAGTCAGCAAGTGGC GGTGGAAAAACTGGTCGTGGAC GCCGTTTCCATTTAGG TGGGTA	SMR11785	*tet*4	*zic3858.9*	3858910 - 3858939 (~82.88')
P12	CGACCGAGGCGCTCGGTACCG TTTTCGACAACGTAATCGCCTGC CCGCGGAATAACATCATTTGGT				
P13	CCTCAAGGGAATGCGCTGACAA ATACGTTGTAATCTATTGATGGA CCGTTTCCATTTAGGTGGGTA	SMR12439	*tet*5	*zic3829.8*	3829822 – 3829836 (~82.25')
P14	CGACGGAGCCGGTGCGGTAAA CATCAATCTCGCCGGGTACGAC TCCGCGGAATAACATCATTTGGT				
P15	CGGTCGCACCATCGCATTACGC GTAGCGCCTTTAATTTGCGCTA GCCGTTTCCATTTAGGTGGGTA	SMR12440	*tet*6	*zib3786.6*	3786668 - 3786676 (~81.3')
P16	TCCAGCATCAGTGGCAAGGCTG GGGCAAACAGCCCGAAGCGGC GACGCGGAATAACATCATTTGG T				
P17	CATATTTCGCATTTGAATATTGG TCAGGATCTCACACCTGCTTCA CCGTTTCCATTTAGGTGGGTA	SMR12453	*tet*7	*zhi3661.4*	3661454 - 3661477 (~78.65')
P18	ACCGCCTGGCAAACTGCGAAAG AGAACTAGCCGGTAGCCCGGCA				

Primer	Sequence (5' - 3')	Strain	Cassette	Allele	Deletion coordinates (Minute position)
	GCGCGGAATAACATCATTTGGT				
P19	CAGCCTTCACGATAGCCACGCT CATACGCTTTACGCTTACCGTTT CCATTTAGGTGGGTA	SMR11694	*tet*9	*zfd2509.2*	2509244 - 2509256 (~53.9')
P20	GGCGCAAAAACCGTTGGTGGCG TCATGATAATGGCTACCCGCGG AATAACATCATTTGGT				
P21	CCATAAGCGCTAACTTAAGGGT TGAACCATCTGAAGAATGCGAC GGCTGGTGGGCTGGTGGGCTG GTGGGGATGAATGTCAGCTAC	SMR11763	I-site B	*zfd2513.5*	2513573 - 2513593 (~53.99')
P22	GTATCCCTGACAGCCTACAAAA CGCAATTGAAGAACGCGAGGCA TATTACCCTGTTATCCCTACGAA ATCTCGTGATGGCAGG				
P23	CTTGAAGCAGCAGGGGATTGAT TGGAATGGTGTTTTTTAGATGTG AGAAATATTTTACCCCCGTTTCC ATTTAGGTGGGTA	SMR12449	t*et*12	*zfd2474.4*	2474465 – 2474539 (~53.15')
P24	ACCTTCATCTATGACCGGATTAA GTCAGGCGACCTGCCAAAAGCC AAAGTTATCCACGGGCGCGGAA TAACATCATTTGGT				
P25	GCTTAGATTTACGCTGTCTTTTG ATCAAATTATTACCATCGGTTGG CTGGTGGGCTGGTGGGCTGGT GGGGATGAATGTCAGCTAC	SMR11768	I-site C	*zie3936.8*	3936888 – 3936907 (~84.55')
P26	CTATACGTTTATCACAATGGGCA TTATTACTGTTGCTTCAGCAGCA TTACCCTGTTATCCCTACGAAAT CTCGTGATGGCAGG				
P27	GGTTAGGGAAAAATGCCTGATA GCGCTTCGCTTATCAGGCCTAC CCCGTTTCCATTTAGGTGGGTA	SMR11769	*tet*13	*zie3954.5*	3954539 – 3954562 (~84.94')
P28	CGGCTACATGAGCCGCTAATTG AGGCATTCTGGAAGATTTTGCC GCGCGGAATAACATCATTTGGT				
P29	GTGCACGAACGGTCCCCTCGCC CCTTTGGGGTGAGGGTTACCGT TTCCATTTAGGTGGGT	SMR12726	*tet*8	*zhc3388.1*	3388124 – 3388141 (~72.78')
P30	GTAATTCATATTGTACTGTTACG TTGTACAAACCTGTGCCCGCGG AATAACATCATTTGG				
P31	GCCGAATGGGCAATTGAACTCT GAAACCGTTGTCTGATGCGCTT CCCGTTTCCATTTAGGTGGGTA	SMR11784	*tet*10	*zdc1532.9*	1532951 - 1532970 (~32.92')
P32	GGTTGCCGGATGCGGCGTAAAC GCCTTATCCGGCATACATTAGC CCGCGGAATAACATCATTTGGT				
P33	CCTTTTTAGTGAATAAATCATCA CTAATTCTATAAGTGTCTGGTCG CTGGTGGGCTGGTGGGCTGGT GGGGATGAATGTCAGCTAC	SMR12725	I-site D	*zdd1542.3*	1542348-1542374 (~33.12')
P34	GAACGTATGTCATGTTTCAACCC TTCAGATCGTGAATCTAAAGGG ATTACCCTGTTATCCCTACGAAA TCTCGTGATGGCAGG				
P35	CGTGAGACGGTTGTTCAACTGA CGGATTTCAGATGATCTCCCGTT	SMR12505	*tet*11	*zfd2502.5*	2502502-2502528 (~53.75')

Primer	Sequence (5' - 3')	Strain	Cassette	Allele	Deletion coordinates (Minute position)
	TCCATTTAGGTGGGT				
P36	GCATGTAGACCGTTAGGCAAAG GACAGAGAAATTGAATCGCGCG GAATAACATCATTTGG				
P37	GACCAGGGCAATGCGCTAATGG CTGGGCGGGAAGGGGATCCGT TGTATCAGATATAGAGTCCGTTT CCATTTAGGTGGGTA	SMR12450	*tet*14	*zfe2523.9*	2523923-2523949 (~54.22')
P38	CGAAGATTTTTACCAGGATGCG ATAATCTACTCCATCAAATTTAA CTTCTGATTGATTACCGCGGAAT AACATCATTTGGT				
P39	CCGTCGCTGGACAAGTTGATGG CGCTGTAAGCGTTTCTGATTCAT TGATTAAAGGCCAGGCCGTTTC CATTTAGGTGGGTA	SMR12459	*tet*15	*zfe2547.5*	2547597-2547638 (~54.72')
P40	GAATTAGCACGATTTCGTAGGC CGGATAAGGCGTTCACGCCGCA TCCGGCGTGTACAACGCGCGGA ATAACATCATTTGGT				
P41	CAATCCCTATGAAAGATTTCATC ACCGAAGCATGGCTAAGCCGTT TCCATTTAGGTGGGT	SMR12494	*tet*16	*zff2572.2*	2572275-2572296 (~55.25')
P42	GCGTCAGGCGACTGTCCGCAG GGAGATGGATCTCTGCGCCCGC GGAATAACATCATTTGG				
P43	GCCAGTCTGGAAACAGGCTGGC TTTTTTTTGCGCGTGTGCGCGG AATAACATCATTTGGT	SMR12480	*tet*2'	*zie3913.1'*	3913117 - 3913122 (~84.06')
P44	GGTTTTATATCAGCAGGATCTAT GTGAACGCTATTCAGGCCGTTT CCATTTAGGTGGGTA				
P45	GATGTCGGTGCACAGATTCGCC AGGCACAACAATGCTAACCCGT TTCCATTTAGGTGGGT	SMR10804	*tet*17	*zie3927.2*	3927225 – 3927226 (~84.35')
P46	CTCTTCGCTGACGGCCAGCATC ACATTAAGCGTATCCAGCCGCG GAATAACATCATTTGG				
P47	CTGAAAGCGATGATGGCGGCAA AACGAGGGAAATAATCAAGGCC GGTGTAGGCTGGAGCTGCTTC	SMR16673	I-site E	*zif3960.3'*	3960326-3960337 (~85.06')
P48	GGCCGCTGGAAGTGCATTAATG AGTAAGTGCCGGATGCGATGCT GATTACCCTGTTATCCCTACATA TGAATATCCTCCTTAG				
P49	CATGCGCATTTGCTGGTGGTGA GTAAGTAGAAATCGGCGGCCGC CGTGTAGGCTGGAGCTGCTTC	SMR16674	I-site F	*zif3981.8'*	3981825-3981831 (~85.54')
P50	CGATCATGTAGGCCGGATAAGG CGTTTACGCCGCATCCGGCAAT CATTACCCTGTTATCCCTACATA TGAATATCCTCCTTAG				
P51	GCGTTATTTGCCGGATTTGGCA TTCATTTGATTATTTCGCGGTGA GTGTAGGCTGGAGCTGCTTC	SMR16675	I-site G	*zig4005.9'*	4005988-4006012 (~86.05')
P52	CATGCGTGAAAAATAATGTCGG ATGCGGCGTAAACGCCTTATCC GATTACCCTGTTATCCCTACATA TGAATATCCTCCTTAG				

Primer	Sequence (5' - 3')	Strain	Cassette	Allele	Deletion coordinates (Minute position)
P53	GAATGGTAATGGGAAAAAATGA TGTCAATCAGATTGCTGACAAC GGTGTAGGCTGGAGCTGCTTC	SMR16700	I-site H	*zih4077.5'*	4077589-4077599 (~87.58')
P54	ACGACTTTGTAGGCCGGATAAG GCGTTCACGCCGCATCCGGCAT GATTACCCTGTTATCCCTACATA TGAATATCCTCCTTAG				
P55	GACCGGATTTCCGAAAAAAGCG CCGCACGGCGCTTTTTTTGTGC CGTGTAGGCTGGAGCTGCTTC	SMR16701	I-site I	*zii4124.8'*	4124854-4124874 (~88.6')
P56	GCGCCACAACTCACCACGGTCG CCAAAGAATGGCCGCTGTTTTC GATTACCCTGTTATCCCTACATA TGAATATCCTCCTTAG				
P57	ACTGGTTACATTTAACGCCACGT TCACTCTTTTGCATCAACAAGAG GATGAATGTCAGCTA	SMR10771	I-site A'	*zie3920.5*'	3920551 – 3920571 (~84.2')
P58	CGATTTTTAGCAGACTGATATTT TCACTAATGACTTATTTTCTGCA TTACCCTGTTATCCCTACGAAAT CTCGTGATGGCAGG				
P59	CATAAGCGCTAACTTAAGGGTT GAACCATCTGAAGAATGCGACG GATGAATGTCAGCTAC	SMR12728	I-site B'	*zfd2513.5*'	2513573 – 2513593 (~53.99')
P60	GTATCCCTGACAGCCTACAAAA CGCAATTGAAGAACGCGAGGCA TTACCCTGTTATCCCTACGAAAT CTCGTGATGGCAGG				

Strains Used in Each Figure

Figure 1. **(B)** DSB *tet1*, SMR10901; CS *tet1*, SMR10900; DSB *tet2*, SMR10866; CS *tet2*, SMR10865; DSB *tet3*, SMR10788; CS *tet3*, SMR10787; DSB *tet4*, SMR12438; CS *tet4*, SMR12437; DSB *tet5*, SMR12446; CS *tet5*, SMR12445; DSB *tet6*, SMR12448; CS *tet6*, SMR12447; DSB *tet7*, SMR12457; CS *tet7*, SMR12456; DSB *tet8*, SMR12732; CS *tet8*, SMR12731; DSB *tet9*, SMR11725; CS *tet9*, SMR11724; DSB *tet10*, SMR11790; CS *tet10*, SMR11789. **(C)** DSB *tet2,* SMR10866; Δ*umuC* DSB *tet2,* SMR14684; Δ*dinB* DSB *tet2,* SMR10868; Δ*dinB* Δ*umuC* DSB *tet2,* SMR14590; Δ*dinB* Δ*polB* DSB *tet2,* SMR12810; DSB *tet4,* SMR14582; Δ*umuC* DSB *tet4,* SMR14688; Δ*dinB* DSB *tet4,* SMR12764; Δ*dinB* Δ*umuC* DSB *tet4,* SMR14594; Δ*dinB* Δ*polB* DSB *tet4,* SMR12814; DSB *tet6,* SMR14586; Δ*umuC* DSB *tet6,* SMR14692; Δ*dinB* DSB *tet6,* SMR13124; Δ*dinB* Δ*umuC* DSB *tet6,* SMR14598; Δ*dinB* Δ*polB* DSB *tet6,* SMR13153; DSB *tet7,* SMR14588; Δ*umuC* DSB *tet7,* SMR14694; Δ*dinB* DSB *tet7,* SMR13126; Δ*dinB* Δ*umuC* DSB *tet7,* SMR14600; Δ*dinB* Δ*polB* DSB *tet7,* SMR13155.

Figure 2. **(B)** DSB *tet9*, SMR11765; CS *tet*9, SMR11764; DSB *tet11*, SMR12750; CS *tet11*, SMR12749; DSB *tet12*, SMR12471; CS *tet12*, SMR12470. **(D)** DSB *tet14*, SMR12482; CS *tet14*, SMR12481; DSB *tet15*, SMR12477; CS *tet15*, SMR12476; DSB *tet16*, SMR12748; CS *tet16*, SMR12747. **(F)** DSB *tet10*, SMR12730; CS *tet10*, SMR12729. **(H)** DSB *tet13*, SMR11778; CS *tet13*, SMR11777. (**I**) DSB *tet17*, SMR10810; No DSB *tet17*, SMR10809; DSB *tet13*, SMR11776; No DSB *tet13*, SMR11775. (**L**) DSB *tet2*, SMR10866; No DSB *tet2*, SMR10865; DSB *tet2'*, SMR12488; No DSB *tet2'*, SMR12487.

Figure 3. (**B**) WT DSB *tet1*, SMR10901; WT DSB *tet2*, SMR10866; WT DSB *tet3*, SMR10788; WT DSB *tet4*, SMR12438; WT DSB *tet5*, SMR12446; WT DSB *tet6*, SMR12448; WT DSB *tet7*, SMR12457; WT DSB *tet8*, SMR12732; WT DSB *tet9*, SMR11725. Δ*recD* DSB *tet1*, SMR14618; Δ*recD* DSB *tet2*, SMR10858; Δ*recD* DSB *tet3*, SMR14620; Δ*recD* DSB *tet4*, SMR14622; Δ*recD* DSB *tet5*, SMR14624; Δ*recD* DSB *tet6*, SMR14626; Δ*recD* DSB *tet7*, SMR14628; Δ*recD* DSB *tet8*, SMR14630; Δ*recD* DSB *tet9*, SMR14632**.** **(D)** WT DSB I-Site E, SMR16729; WT DSB I-Site F, SMR16731; WT DSB I-Site G, SMR16733; WT DSB I-Site H, SMR16735; WT DSB I-Site I, SMR16737. Δ*recD* DSB I-Site E, SMR16746; Δ*recD* DSB I-Site F, SMR16748; Δ*recD* DSB I-Site G, SMR16750; Δ*recD* DSB I-Site H, SMR16752; Δ*recD* DSB I-Site I, SMR16754.

Figure 4. **(B)** Chi^+ No-DSB, SMR10799; Chi^+ DSB, SMR10800; Chi° No-DSB, SMR12488; Chi° DSB, SMR12489. **(D)** Chi^+ No-DSB, SMR12749; Chi^+ DSB, SMR12750; Chi° No-DSB, SMR12753; Chi° DSB, SMR12754. **(F)** Chi^+ No-DSB, SMR12481; Chi^+ DSB, SMR12482; Chi° No-DSB, SMR12735; Chi° DSB, SMR12736. All No-DSB strains are the cutsite-only control.