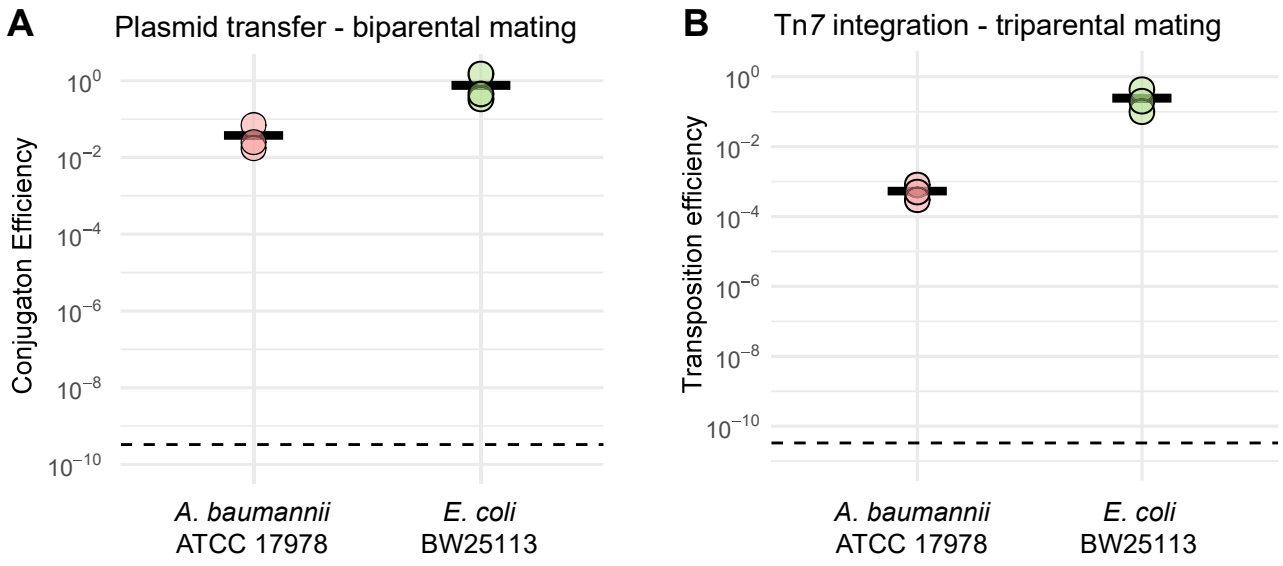
Figure S1 Conjugation and transposition efficiencies. (A) Dot plot of conjugation efficiency of the replicative vector to the recipient strain (*A. baumannii* ATCC 17978 or *E. coli* BW25113) after ~3-hour incubations. Bars represent mean efficiencies (n=3). Dashed line is drawn at the limit of detection, defined by the maximum number of colony-forming units (CFUs) observed on non-selective plates in each experiment. **(B)** Dot plot showing transposition efficiency of the Tn*7* transposon into the att_{Tn7} site of *A. baumannii* ATCC 17978 or *E. coli* BW25113 after ~4-hour incubation with donor strains, one carrying the Tn*7* transposon vector and another carrying the Tn*7* transposase vector. Bars represent mean efficiencies (n=3); dashed line shows limit of detection. **(C)** Table showing instances of carbenicillin-resistant (CarbR) transconjugants of 40 isolates tested for Tn*7* co-integrates in *A. baumannii* and *E. coli*.

Figure S2 Titration of P_{abstBR} expression. Expression from **(A)** the replicative plasmid vector or **(B)** the Tn*7* transposon. Plots shown are normalized sfGFP levels expressed from P_{abstBR} across IPTG concentrations for *A. baumannii* ATCC 19606 and *A. baumannii* AB5075. Error bars represent standard deviation (n=3 for replicative vector, n=6 for Tn*7* transposon).

S1

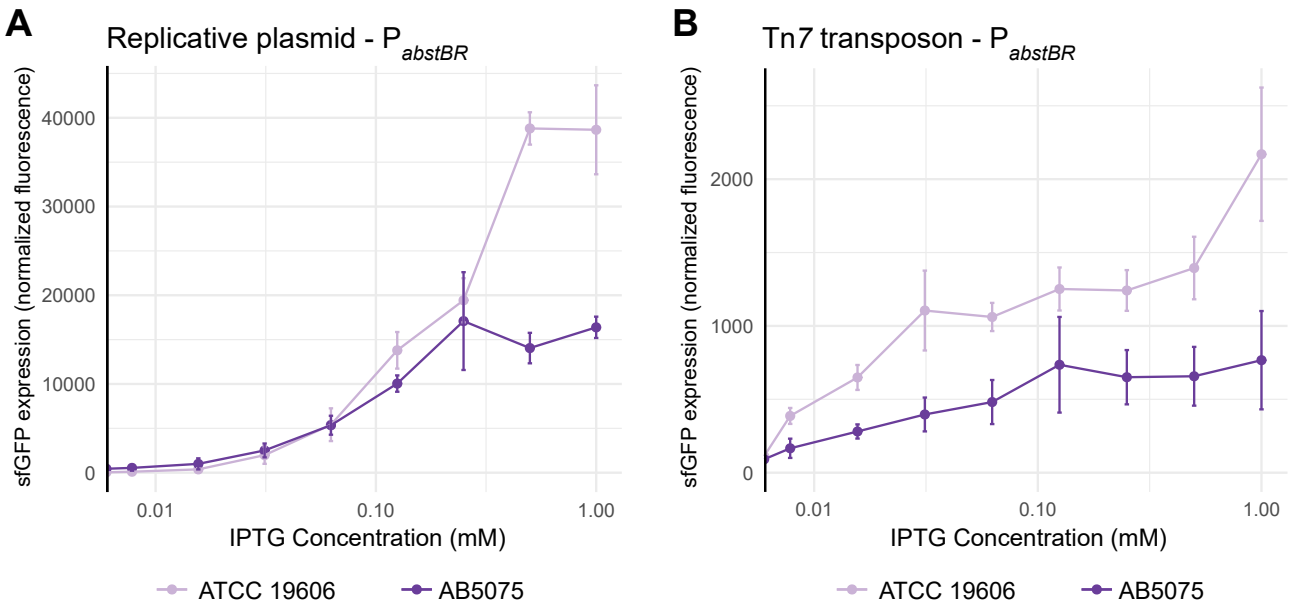


C

Co-integrates: CarbR Tn*7* transconjugants

	Strain	
	A. baumannii ATCC 17978	*E. coli* BW25113
Tn*7* vector - empty	2/40	0/40
Tn*7* vector - sfGFP	3/40	1/40

S2


A
Replicative plasmid - P abstBR
sfGFP expression (normalized fluorescence)
40000
30000
20000
10000
0
0.01
0.10
1.00
IPTG Concentration (mM)
ATCC 19606
AB5075
B
Tn7 transposon - P abstBR
sfGFP expression (normalized fluorescence)
2000
1000
0
0.01
0.10
1.00
IPTG Concentration (mM)
ATCC 19606
AB5075

Supplemental Table S1 Strains

Key

Term	Description
Strain #	JMP lab accession number
Description	Descriptive name, genotype, plasmids, construction details, phenotype, usage, etc
Source	Strains produced in this study unless otherwise noted

Strain #	Description[a]	Source
sJMP3053	*Escherichia coli* (derived from BW25141) pir+, recAI cloning strain with anti-CRISPR (attTn7::acrIIA4); Δ*(araD-araB)*567, Δ*lacZ4787* (::*rrnB-3*), Δ(*phoB-phoR*)580, l-, *galU95*, Δ*uidA3*::pir+, recA1, endA9(del-ins)::FRT, *rph-1*, Δ(*rhaD-rhaB*)*568*, *hsdR514, attTn7::acrIIA4*	Ward, PMID: 38126769
sJMP3075	*Escherichia coli* MG1655 wild-type (sJMP163, CAG80011)	Carol Gross, UCSF
sJMP3076	*Escherichia coli* BW25113 (sJMP6, CAG74538); F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514	Carol Gross, UCSF
sJMP3257	*Escherichia coli* (derived from WM6026) pir+, dap- mating strain with anti-CRISPR (attTn7::acrIIA4); laclq, rrnB3, DElacZ4787, hsdR514, DE(araBAD)567, DE(rhaBAD)568, rph-1 att-lambda::pAE12-del (oriR6K/cat::frt5), del 4229(dapA)::frt(DAP-), del(endA)::frt, uidA(delMluI)::pir(wt), attHK::pJK1006::del1/2(del oriR6K-cat::frt5, del trfA::frt), attTn7::acrIIA4	Banta, PMID: 38496613
sJMP3261	*Escherichia coli* sJMP3257 mating strain with plasmid pTn7C1 (pJMP1039) transposase expression; ampR, dap-	Banta, PMID: 38496613
sJMP3329	*Acinetobacter baumannii* ATCC 19606 WT	ATCC
sJMP3348	*Acinetobacter baumannii* ATCC 17978 WT	ATCC
sJMP3518	*Acinetobacter baumannii* AB5075 WT	Colin Manoil, UW
sJMP3683	*E. coli* sJMP3257 mating strain with plasmid pJMP3649 (Ptrc EV); kanR, dap-	this study
sJMP3684	*E. coli* sJMP3257 mating strain with plasmid pJMP3650 (Ptrc sfGFP); kanR, dap-	this study
sJMP3685	*E. coli* sJMP3257 mating strain with plasmid pJMP3651 (Pabst EV); kanR, dap-	this study
sJMP3686	*E. coli* sJMP3257 mating strain with plasmid pJMP3652 (Pabst sfGFP); kanR, dap-	this study
sJMP3687	*E. coli* sJMP3257 mating strain with plasmid pJMP3653 (PabstBR EV kan); kanR, dap-	this study
sJMP3688	*E. coli* sJMP3257 mating strain with plasmid pJMP3654 (PabstBR sfGFP kan); kanR, dap-	this study
sJMP3689	*E. coli* sJMP3257 mating strain with plasmid pJMP3664 (PabstBR EV apr); aprR, dap-	this study
sJMP3690	*E. coli* sJMP3257 mating strain with plasmid pJMP3666 (PabstBR sfGFP apr); aprR, dap-	this study
sJMP3691	*E. coli* sJMP3257 mating strain with plasmid pJMP3665 (PabstBR EV hyg); hygR, dap-	this study
sJMP3692	*E. coli* sJMP3257 mating strain with plasmid pJMP3667 (PabstBR sfGFP hyg); hygR, dap-	this study
sJMP3715	*A. baumannii* 17978 (sJMP3348) with plasmid pJMP3649 (Ptrc EV); kanR	this study
sJMP3716	*A. baumannii* 17978 (sJMP3348) with plasmid pJMP3650 (Ptrc sfGFP); kanR	this study
sJMP3717	*A. baumannii* 17978 (sJMP3348) with plasmid pJMP3651 (Pabst EV); kanR	this study
sJMP3718	*A. baumannii* 17978 (sJMP3348) with plasmid pJMP3652 (Pabst sfGFP); kanR	this study
sJMP3719	*A. baumannii* 17978 (sJMP3348) with plasmid pJMP3653 (PabstBR EV); kanR	this study
sJMP3720	*A. baumannii* 17978 (sJMP3348) with plasmid pJMP3654 (PabstBR sfGFP); kanR	this study
sJMP3721	*A. baumannii* 19606 (sJMP3329) with plasmid pJMP3664 (PabstBR EV); aprR	this study
sJMP3722	*A. baumannii* 19606 (sJMP3329) with plasmid pJMP3666 (PabstBR sfGFP); aprR	this study
sJMP3725	*A. baumannii* AB5075 (sJMP3518) with plasmid pJMP3665 (PabstBR EV); hygR	this study
sJMP3726	*A. baumannii* AB5075 (sJMP3518) with plasmid pJMP3667 (PabstBR sfGFP); hygR	this study
sJMP3733	*E. coli* BW25113 (sJMP3076) with plasmid pJMP3653 (PabstBR EV); kanR	this study
sJMP3734	*E. coli* BW25113 (sJMP3076) with plasmid pJMP3654 (PabstBR sfGFP); kanR	this study
sJMP3844	*A. baumannii* 17978 (sJMP3348) with attTn7::PrpoE-mRFP reporter (Tn7 from pJMP3748; rpoE promoter sequence from E. coli), aprR	this study
sJMP3845	*E. coli* (sJMP3075) with attTn7::PrpoE-mRFP reporter (Tn7 from pJMP3748; rpoE promoter sequence from E. coli), aprR	this study
sJMP4061	*E. coli* mating strain with helper plasmid pEVS104 (sJMP2935; helper strain); kanR, dap-	Ward, PMID: 38126769
sJMP12051	*A. baumannii* 17978 (sJMP3348) with attTn7::PabstBR-sfGFP (Tn7 from pJMP12049); aprR	this study
sJMP12053	*A. baumannii* 17978 (sJMP3348) with attTn7::PabstBR-empty (Tn7 from pJMP12042); aprR	this study
sJMP12055	*A. baumannii* 19606 (sJMP3329) with attTn7::PabstBR-sfGFP (Tn7 from pJMP12049); aprR	this study
sJMP12057	*A. baumannii* 19606 (sJMP3329) with attTn7::PabstBR-empty (Tn7 from pJMP12042); aprR	this study
sJMP12059	*E. coli* BW25113 (sJMP3076) with attTn7::PabstBR-sfGFP (Tn7 from pJMP12049); aprR	this study
sJMP12061	*E. coli* BW25113 (sJMP3076) with attTn7::PabstBR-empty (Tn7 from pJMP12042); aprR	this study
sJMP12063	*E. coli* sJMP3257 mating strain with plasmid pJMP12042 (Tn7 PabstBR EV); ampR, aprR, dap-	this study
sJMP12065	*E. coli* sJMP3257 mating strain with plasmid pJMP12049 (Tn7 PabstBR sfGFP); ampR, aprR, dap-	this study
sJMP12070	*E. coli* mating strain with plasmid pJMP3853 (PabstBR RpoE OE vector); kanR, dap-	this study
sJMP12074	(pJMP3853), aprR, kanR	this study
sJMP12076	kanR	this study
sJMP12083	*A. baumannii* AB5075 (sJMP3518) with attTn7::PabstBR-empty (Tn7 from pJMP12042); aprR	this study
sJMP12085	A. baumannii AB5075 (sJMP3518) with attTn7::PabstBR-sfGFP (Tn7 from pJMP12049); aprR	this study

[a]ampR, ampicillin resistant; aprR, apramycin resistant; kanR, kanamycin resistant; hygR, hygromycin resistant; dap-, requires diaminopimelic acid.

Supplemental Table S2 Plasmids

Key

Term	Description
Plasmid #	JMP lab accession number
Description	Descriptive name
Addgene #	Addgene accession number (https://www.addgene.org/)
Construction/note	Plasmid construction notes, usage, or further details
Promoter-gene	Promoter used for expressing specific gene or reporter (if applicable)
Resistance	Antibiotic resistance cassette(s)
Source	plasmids produced in this study unless otherwise noted

Key plasmids from this study are shown in bold

Plasmid #	Description	Addgene #	Construction/notes	Promoter-gene	Resistance[a]	Source
pJMP0631	Tiny Tn7 vector		pTinyTn7 plasmid, R6k ori vector with Tn7 transposon with kanR cassette	na	ampR, kanR	Hall, PMID: 37662258
pJMP1039	pTn7C1	119239	Tn7 transposase expression	na	ampR	Peters, PMID: 30617347
pJMP2748	vector that contains GFP		sfGFP is on this vector for amplification	na	ampR, gentR	Ward, PMID: 38126769
pJMP3067	pTrc99a		pTrc99a expression plasmid, pBR322 ori expression vector containing IPTG inducible trc promoter	Ptrc-empty	ampR	Rhodius, PMID:16336047
pJMP3262	pJQ200SK with p15A ori	78497	pJQ200SK, gent-sacB vector containing E. coli p15A origin of replication	na	gentR	Quandt, PMID: 8486283
pJMP3341	Tiny Tn7 with NcoI site removed		Tiny-Tn7 plasmid; HiFi assembly to delete NcoI sites from pJMP631 with o1255/1258 and o1256/1257 amplified from pJMP631	na	ampR, kanR	this study
pJMP3347	pSGAb-km with pWH1266 ori	121999	plasmid containing pWH1266 A. baumannii origin of replication	na	kanR	Wang, PMID: 31548010
pJMP3352	Old Ptrc-EV expression vector, R6k version		overexpression plasmid containing pTrc99a expression system, Ecoli R6K gamma origin, Abaumannii pWH1266 origin; (requires pir to replicate in E. coli)	Ptrc-empty	kanR	this study
pJMP3361	Old Ptrc-GFP expression vector, R6k version		overexpression plasmid containing Ptrc promoter expressing GFP, Ecoli R6K gamma origin, Abaumannii pWH1266 origin; HiFi assembled sfGFP gene amplified from pJMP2748 with oJMP1253/oMP1254 into pJMP3352 cut with NcoI/BamHI; (requires pir to replicate in E. coli)	Ptrc-GFP	kanR	this study
pJMP3407	Old Pabst-EV expression vector, R6k version		overexpression plasmid containing Pabst promoter, Ecoli R6K ori, Abaumannii pWH1266 ori; construced by PCR amplifying pJMP3352 with oJMP2040 and oJMP2041, and HiFi assembling with gblock oJMP2039 and pJMP3352 digested with EcoRI (used larger piece from digest via gel purification); (requires pir to replicate in E. coli)	Pabst-empty	kanR	this study
pJMP3434	Old Pabst-GFP expression vector, R6k version		overexpression plasmid containing Pabst promoter expressing GFP, Ecoli R6K gamma origin, Abaumannii pWH1266 origin; HiFi assembled sfGFP gene amplified from pJMP2748 with oJMP1253/oMP1254 into pJMP3407 cut with NcoI/BamHI; (requires pir to replicate in E. coli)	Pabst-GFP	kanR	this study
pJMP3649	**Ptrc EV kan - replicative vector used in this study**		**Ptrc OE empty vector with p15A(ACYC) and pWH1266 origins; constructed by digesting pJMP3352 with AscI/PmeI, and HiFi assembling with PCR product of oJMP2134 and oJMP2135 amplified from pJQ200SK**	**Ptrc-empty**	**kanR**	**this study**
pJMP3650	Ptrc GFP kan - replicative vector used in this study		Ptrc-GFP expression vector with p15A(ACYC) and pWH1266 origins; constructed by digesting pJMP3361 with AscI/PmeI, and HiFi assembling with PCR product of oJMP2134 and oJMP2135 amplified from pJQ200SK	Ptrc-GFP	kanR	this study
pJMP3651	**Pabst EV kan - replicative vector used in this study**		**Pabst OE empty vector with p15A(ACYC) and pWH1266 origins; constructed by digesting pJMP3407 with AscI/PmeI, and HiFi assembling with PCR product of oJMP2134 and oJMP2135 amplified from pJQ200SK**	**Pabst-empty**	**kanR**	**this study**
pJMP3652	Pabst GFP kan - replicative vector used in this study		Pabst-GFP expression vector with p15A(ACYC) and pWH1266 origins; constructed by digesting pJMP3434 with AscI/PmeI, and HiFi assembling with PCR product of oJMP2134 and oJMP2135 amplified from pJQ200SK	Pabst-GFP	kanR	this study
pJMP3653	**PabstBR EV kan - replicative vector used in this study**	*	**PabstBR OE empty vector with p15A(ACYC) and pWH1266 origins; constructed by digesting pJMP4481 with AscI/PmeI, and HiFi assembling with PCR product of oJMP2134 and oJMP2135 amplified from pJQ200SK**	**PabstBR-empty**	**kanR**	**this study**
pJMP3654	**PabstBR GFP kan - replicative vector used in this study**	*	**PabstBR-GFP epxression vector with p15A(ACYC) and pWH1266 origins; constructed by digesting pJMP4491 with AscI/PmeI, and HiFi assembling with PCR product of oJMP2134 and oJMP2135 amplified from pJQ200SK**	**PabstBR-GFP**	**kanR**	**this study**
pJMP3664	**PabstBR EV apr - replicative vector used in this study**	*	**Apramycin version of pJMP3653 (PabstBR-empty vector); digested pJMP3653 with XhoI, HiFi assembled with gblock oJMP1946**	**PabstBR-empty**	**aprR**	**this study**
pJMP3665	**PabstBR EV hyg - replicative vector used in this study**	*	**Hygromycin version of pJMP3653 (PabstBR-empty vector); digested pJMP3653 with XhoI, HiFi assembled with gblock oJMP2117**	**PabstBR-GFP**	**hygR**	**this study**
pJMP3666	PabstBR GFP apr - replicative vector used in this study	*	Apramycin version of pJMP3654 (PabstBR-empty vector); digested pJMP3654 with XhoI, HiFi assembled with gblock oJMP1946	PabstBR-empty	aprR	this study
pJMP3667	PabstBR GFP hyg - replicative vector used in this study	*	Hygromycin version of pJMP3654 (PabstBR-empty vector); digested pJMP3654 with XhoI, HiFi assembled with gblock oJMP2117	PabstBR-GFP	hygR	this study
pJMP3748	Tn7 PrpoE-mRFP reporter vector		Integrative expression vector with PrpoE-mRFP reporter on Tn7 transposon; derived from pJMP8602 by adding PrpoE in front of mRFP reporter using oligos oJMP2369 and oJMP2370	PrpoE-mRFP	ampR, aprR	this study
pJMP3853	PabstBR rpoE OE vector kan		PabstBR-RpoE expression vector, parent pJMP3653; HiFi assembled rpoE gene amplified from E. coli sJMP3075 gDNA with oJMP2634 and oJMP2635 into pJMP3653 digested with NcoI/BamHI	PabstBR-RpoE	kanR	this study
pJMP4003	pEVS104 helper plasmid	207393	pEVS104(R6K ori, KanR, Tra+)	na	kanR	Stabb, PMID: 12474404
pJMP4481	Old PabstBR-EV expression vector, R6k version		Overexpression plasmid containing PabstBR promoter, Ecoli R6K ori, Abaumannii pWH1266 ori; construced by site-directed mutagenesis using pJMP3407 and oJMP2167; (requires pir to replicate in E. coli)	PabstBR-empty	kanR	this study
pJMP4491	Old PabstBR-GFP expression vector, R6k version		Overexpression plasmid containing PabstBR promoter expressing GFP, Ecoli R6K gamma origin, Abaumannii pWH1266 origin; HiFi assembled sfGFP gene amplified from pJMP2748 with oJMP1253/oMP1254 into pJMP4481 cut with NcoI/BamHI; (requires pir to replicate in E. coli)	PabstBR-GFP	kanR	this study
pJMP8602	Old vector containing Tn7 base		Tn7 expression vector with mRFP reporter on Tn7 transposon	none-mRFP	kanR	Hall, PMID: 37662258
pJMP12042	**Tn7 PabstBR EV apr - integrative vector used in this study**	*	**Integrative expression vector with PabstBR empty vector on Tn7 transposon; constructed by amplifying pJMP3653 with oJMP2539 and oJMP2540, pJMP8602 with oJMP2541 and oJMP2542, pJMP3352 with oJMP2543 and oJMP2544, and HiFi assembling with pJMP3539 digested with SpeI/NotI**	**PabstBR-empty**	**ampR, aprR**	**this study**
pJMP12049	**Tn7 PabstBR GFP apr - integrative vector used in this study**	*	**Integrative expression vector with PabstBR-GFP on Tn7 transposon; constructed by digesting pJMP12042 with NcoI/BamHI, and HiFi assembling with PCR product of oJMP1254 and oJMP2078 amplified from pJMP2748**	**PabstBR-GFP**	**ampR, aprR**	**this study**
pJMP12068	**Tn7 PabstBR EV kan - integrative vector used in this study**	*	**Kanamycin version of pJMP12042 (Tn7-PabstBR-empty vector); HiFi assembled PCR product of pJMP3653 with oJMP0193 and oJMP0194 into pJMP12042 digested with XhoI**	**PabstBR-empty**	**ampR, kanR**	**this study**
pJMP12089	**Tn7 PabstBR EV hyg - integrative vector used in this study**	*	**Hygromycin version of pJMP12042 (Tn7-PabstBR-empty vector); digested pJMP12042 with XhoI, HiFi assembled with gblock oJMP2117**	**PabstBR-empty**	**ampR, hygR**	**this study**

[a]ampR, ampicillin resistant; aprR, apramycin resistant; gentR, gentamycin resistant; kanR, kanamycin resistant

*to be submitted to Addgene

Supplemental Table S3 Oligonucleotides and Synthetic DNA

Key

Term	Description
Oligo #	JMP lab accession number
Sequence	Nucleotide sequence
Usage	Procedure using this oligonucleotide/synthetic DNA

Oligo #	Sequence[a]	Usage
oJMP0193	TCCTGAACGGCCATAAGAAC	amplifying kanR marker for swapping A
oJMP0194	GAGCGCTTTTGAAGCTGATG	amplifying kanR marker for swapping B
oJMP1253	CGGATAACAATTTCACACAGGAAACAGACCATGAGCAAAGGAGAAGAACT	amplify GFP A
oJMP1254	TGCATGCCTGCAGGTCGACTCTAGAGGATCTTTGTAGAGCTCATCCATGC	amplify GFP B
oJMP1255	TTCGGCAAGCAGGCATCGCCGTGGGTCACGACGAGATCCT	remove NcoI sites from pJMP631 A
oJMP1256	GAGGATCTCGTCGTGACCCACGGCGATGCCTGCTTGCCGA	remove NcoI sites from pJMP631 C
oJMP1257	CTGACATGGGAATTAGCCACGGCATCACAGTATCGTGATG	remove NcoI sites from pJMP631 D
oJMP1258	ATCACGATACTGTGATGCCGTGGCTAATTCCCATGTCAGC	remove NcoI sites from pJMP631 B
oJMP1946	TCCTGAACGGCCATAAGAACGAAGGCTGTCTGTTGAACTCTCGAGCCTTGTCGCCTTGCGTATAATATTTGCTCA	apramycin (aprR) gblock
oJMP2039	AGCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAAGACGTCTCATTAATCAGATAAAATATTATAAATGTC	making Pabst replicative vector gblock
oJMP2040	TGATACCTCTGGCTCAGCG	making Pabst replicative vector A
oJMP2041	TTTACGACCTGCACAGCCAT	making Pabst replicative vector B
oJMP2117	TCCTGAACGGCCATAAGAACGAAGGCTGTCTGTTGAACTCTCGAGCCTTGTCGCCTTGCGTATAATATTTGCTCA	hygromycin (hygR) gblock
oJMP2134	GCCTTCCGTTTGGGCCTAGAATTCCGGTTTAACAGGGAAGTGAGAGGGC	swapping to p15A ori A
oJMP2135	TGGCTTGCAGCCCTTATGTCCTCAGGCGCGCACAAAACAGCAGGGAAGC	swapping to p15A ori B
oJMP2167	CAAGAAGATCATCTTATTAATCAGATAAAATATTGACAATGTGAGCGGATAACAAG	making PabstBR Quikchange primer
oJMP2369	CTAGAAACAAAAACAGATGCGTTACGGAACTTTACAAAAACGAGACACTCTAACCCTTTG	PrpoE cloning A
oJMP2370	TCGTCAAAGGGTTAGAGTGTCTCGTTTTTGTAAAGTTCCGTAACGCATCTGTTTTTGTTT	PrpoE cloning B
oJMP2539	CACGCCGCTTTTTTTACGTCTGCAGACTAGTCAGGCAGCCATCGGAAGCT	amplify MCS A
oJMP2540	TGTCGACTAGGCCATGATGCTCATTCTGTGAAAAGGCCATCCGTCAGGAT	amplify MCS B
oJMP2541	CACAGAATGAGCATCATGGC	amplify Tn7 cassette A
oJMP2542	GGGTCAGTGAGCGAGGAAG	amplify Tn7 cassette B
oJMP2543	TTTAAAACTTCATTTTTAATTTTTGCGGCCGATCTGAAGATCAGCAGTTC	amplify R6k and oriT A
oJMP2544	GGGCTCTTCCGCTTCCTCGCTCACTGACCCTATCAGAGCTTATCGGCCAG	amplify R6k and oriT B

[a]all sequences are 5'-3'