

**Supplementary Table 1.** Primers used in this study.

Primer	Sequence <sup>a</sup>
Ex18-Up	ataaacaataggggtccgcgcacattccccgaaaagtGGGTAATAACTGATATAATTAATT
Ex18-Dn	ttttataggttaatgcatgataataatggttcttagaGATCGCTTGCTGTAACCTTA
F-Psac	accctatcggtgtgaaataccgcacagatgcgtaaggagaaaataGAGTGACCATACCTCTCA
R-Psac/lacZ	ctggcgtaatagcgaagag
F-Ap-to-Gm	gattttggtcatgagattatcaaaaaggatcttcacctagatATGAGTAACTTGGTCTGACAA
R-Ap-to-Gm	tttcggggaaatgtgctggaaccctattgttttttGAAGAGTATGAGTATTCAAC
F-V204-kan	acagatgcgtaaggagaaaatagagtgcaccatacctctcagGTGGATCTGATATCACCTA
R-V204-kan	aggcggattaccctggcgtccaaccagcggcaccagcggcTCGACAACCCTTAATATAAC
F-oriT-V204	ctaaactcacaattagagcttcaatttaattatatacagttatt <b>cacgtg</b> GAGCTTATCGGCCAGCCT
R-oriT-V204	AGGTCTAGAGATCTGTTTAGCTTGCCCTCGTCCCCGCCGGGTCA <b>agccggc</b> ggtta aggtatactttccgct
F-pBAD-HQ/EP	ttcgtattaccgcagctggcgaaaaggggatgtgctgcaaggATGCCTGGCAGTTTATGGC
R-pBAD-HQ/EP	cgcctctccccgcgcttggccgattcattaatgcagctggGCTCATGTTTGACAGCTTAAT
F-p15-HQ/EP	aggaaagaacatgtgagcaaaagccagcaaaagccaggaaccgtCAGCTGCCGCATAAGATGA
R-p15-HQ/EP	cacctactccaacatcag
F--gfp2-pBAD	aatcttctctatccgcaaacagccaagcttgcagctgcag <b>actagt</b> CTATTTGTATAGTTCATCCATG
R--gfp2-pBAD	acccttttttgggctagcgaattcagctcggtagccggggaaggagatatacatATGAGTAAAGGAGAAG AACTT
F-rk2-ep	cccggcgggacgaggaagcaactaaacagatctctagacctGGCCGCCGGCGTTGTGGATAC
R-rk2-ep	gcctgaaaggcagccgggctgtggccacggcctctagtttaataaTGCAGGTGGCTGCTGAAC
R-clpX-int	gatgccacgatcctcgcctgctggcgaagatcactctagCTCGAGACCGGCAAACGCCCT ACAAAC
F-clpX-int	tctaagaaccattattatcatgacattaacctataaaaa <b>aggcctctcgag</b> ACGGCAAGCTGCTGTATTG
R-rhlB-gfp	atcgcaactctactgtttctccatacccgttttttggg <b>aggagg</b> aaaaATGCACGCCATCCTCATCG

F-rhlB-gfp ttgggacaactccagtgaaaagtctctcttactcatGGACGCAGCCTTCAGCCATC

F-lacZ-pBAD ttaatctgtatcaggctgaaaatctctctcatccgccaagtTTCGCCATTCAGGCTGCGC

R-lacZ-pBAD cgcttttatcgcaactcttactgtttctccatacccgtagg**aggaaaaa**ATGACCATGATTACGCCAAG

F-DsRed ggcaaattctgtttatcagaccgcttctgcgttctgattCACCAGACAAGTTGGTAATG

R-DsRed tacgcaaaccgctctccccgcgctggccgattcattaTCATTAATGCAGCTGGCACG

F-Ds-Hc gaccggcgctcagttggaattctagagtcgcccgcctctGTTGGCCTTCTCGGGCAGG

R-Ds-Hc caggtcgactctagaggatccccgggtaccggcgccaccATGGTGAGCGGCCTGCTG

F-rhlB ggccagtgccaagcttgcctgcaggtcgactctagaggatcct**ca**GGACGCAGCCTTCAGCCATC

R-rhlB gctatgacatgattacgaattcgagctcggtaccggggatcCAGAGCCACGTGCATTTTCATC

F-rhlB-kan gttgatctccggcgcgaggtgcatccagcttgaagcgctcgatCCAGCTGAAGCTTCGTACGC

R-rhlB-kan CGAACTGACCTACCGCCGGACCATGGGCGATCCGCGCCTGTGGGA<sup>gcat</sup>  
aggccactagtggatctg

F-3HA ttaatctgtatcaggctgaaaatctctctcatccgcaaTTAGCACTGAGCAGCGTAATCTG

R-3HA catggcatgatgagctctacaagccggcTACCCATACGATGTTTCCTGAC

F-gfpC2 gtcaggaacatcgtatgggtaGCCGGCTTTGTAGAGCTCATCCATGCCATG

R-gfpC ggccaacacttgcactactctgactTATGGTGTTCAATGCTTTTCC

F-gfpN ggaaaagcattgaacaccata**agtcag**AGTAGTGACAAGTGTGGCC

R-gfpN2 aatagattcaagttagttg**gaaggaaaaaa**ATGAGTAAAGGAGAAGAAGACTTTTCACTGG

F-XYL2 tctctcttactcat**tttttttctctc**CAAACCTAATTGAATCTATTATAACAG

R-XYL cgctctccccgcgctggccgattcattaatgcagctggTGGCATTCTACGACTATAAC

LAPD1 for P72 ctctctactgtttccataccggttttttgggtagcgaaGGTCGAGACGACTAACTA

LAPD2 for P72 aatcttctctcatccgcaaacagccaagcttgcctgCTCAGTCAGAAGGATAAAAAGACC

lapD C-term deletion aatcttctctcatccgcaaacagccaagcttgcctgTCAGTGATCGTCACCGACTGG

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a. Lower case letters correspond to regions of homology for targeting recombination by yeast, Upper case letters are regions for PCR-amplification, Bold letters are added sequence including restriction sites and ribosome binding sequences.

**Supplementary Table 2.** Vector construction.

<b>Construct</b>	<b>Parental Construct</b>	<b>Gap Enzyme</b>	<b>Template</b>	<b>Primers</b>
pMQ30	pEX18-Gm	AatII	pYC2-CT	Ex18-Up, Ex18-Dn
pMQ75	pMQ30	BglIII	pVEX15a	F-p15-HQ/EP, R-p15-HQ/EP
pMQ84	pMQ30	<i>MspI, MscI</i>	PA14 chromosome	R-clpX-int, F-clpX-int
pMQ87 <sup>a</sup>	pMQ84	<i>XhoI-XhoI</i> <sup>b</sup>	none	none
pMQ89 <sup>a</sup>	pMQ87	<i>StuI-StuI</i> <sup>b</sup>	none	none
pV204	pRS416	<i>NgoMIV</i>	pUCP20	F-Psac, R-Psac
pV204-2μ	pRS426	<i>NgoMIV</i>	pUCP20	F-Psac, R-Psac
pMQ51	pV204-2μ	<i>NarI</i>	pUG6	F-V204-kan R-V204-kan
pMQ52	pV204	<i>NarI</i>	pUG6	F-V204-kan R-V204-kan
pMQ61	pMQ51	<i>BcgI</i>	pEX18-Gm	F-amp-to-gm, R-amp-to-gm
pMQ56	pMQ52	<i>MluI</i>	mini-CTX	F-oriT-V204 R-oriT-V204
pMQ57 <sup>a</sup>	pMQ56	<i>PmlI-PmlI</i> <sup>b</sup>	none	none
pMQ64	pMQ61	<i>MluI</i>	mini-CTX	F-oriT-V204

R-oriT-V204

pMQ65 <sup>a</sup>	pMQ64	<i>PmlI-XmnI</i> <sup>b</sup>	none	none
pMQ70	pMQ56	<i>SmaI</i>	pBAD18	F-pBAD-HQ/EP R-pBAD-HQ/EP
pMQ71	pMQ61	<i>SmaI</i>	pBAD18	F-pBAD-HQ/EP R-pBAD-HQ/EP
pMQ72	pMQ64	<i>SmaI</i>	pBAD18	F-pBAD-HQ/EP, R- pBAD-HQ/EP
pMQ90 <sup>a</sup>	pMQ70	<i>PmlI-PmlI</i> <sup>b</sup>	none	none
pMQ91	pMQ70	<i>SmaI</i>	pRS415	F-lacZ-pBAD, R- lacZ-pBAD
pMQ78	pMQ70	<i>SmaI</i>	pGFPmut3	F-gfp2-pBAD, R- gfp2-pBAD
pMQ79	pMQ71	<i>SmaI</i>	pGFPmut3	F-gfp2-pBAD, R- gfp2-pBAD
pMQ80	pMQ72	<i>SmaI</i>	pGFPmut3	F-gfp2-pBAD, R- gfp2-pBAD
pMQ95	pMQ70	<i>HpaI</i>	pJB785TTKm1	F-rk2-ep, R-rk2-ep
pMQ97	pMQ72	<i>HpaI</i>	pJB785TTKm1	F-rk2-ep, R-rk2-ep

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a. vector not made by gap repair

b. restriction fragment removed from parental vector

**Supplementary Table 3.** Bacterial host-range of vectors from this study<sup>1</sup>.

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<b><u>RK2 replicon</u></b>	<b><u>pRO1600 replicon</u></b>	<b><u>p15a/ColE1</u></b>
<i>Acetobacter xylinum</i>	<i>Pseudomonas sp.</i>	<i>Enterobacteriaceae</i>
<i>Achromobacter parvulus</i>	<i>Klebsiella sp.</i>	
<i>Acinetobacter spp.</i>		
<i>Aeromonas spp.</i>		
<i>Agrobacterium spp.</i>		
<i>Alcaligenes spp.</i>		
<i>Anabaena spp.</i>		
<i>Azospirillum brasilense</i>		
<i>Azotobacter spp.</i>		
<i>Bartonella spp.</i>		
<i>Bordetella spp.</i>		
<i>Caulobacter spp.</i>		
<i>Clavobacter xyli</i>		
<i>Enterobacteriaceae</i>		
<i>Haemophilus influenzae</i>		
<i>Hypomicrobium X</i>		
<i>Legionella pneumophila</i>		
<i>Klebsiella spp.</i>		
<i>Methylophilus methylotrophus</i>		
<i>Methylosinus trichosporium</i>		

*Myxococcus xanthus*

*Neisseria spp.*

*Paracoccus denitrificans*

*Proteus spp.*

*Pseudomonas spp.*

*Rhizobium spp.*

*Rhodopseudomonas spp.*

*Rhodospirillum spp.*

*Salmonella spp.*

*Serratia marcescens*

*Thiobacillus spp.*

*Vibrio cholerae*

*Xanthomonas spp.*

*Yersinia enterocolitica*

*Zymomonas mobilis*

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1. list is from Thomas and Helinski (1989) Promiscuous plasmids of Gram-Negative Bacteria, p1-25, and Blatny, et al. (1997) Plasmid 38:35-51.