Supporting information

Antibiotic combinations that enable one-step, targeted mutagenesis of chromosomal genes

Wonsik Lee¹, Truc Do¹, Ge Zhang^{1,2}, Daniel Kahne², Timothy C. Meredith^{1,3} and Suzanne Walker^{1*}

¹Department of Microbiology and Immunobiology, Harvard Medical School, Boston, 4 Blackfan Circle Boston, MA 02115, Massachusetts, 02115, United States

²Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Cambridge, Massachusetts, 02138, United States

³Department of Biochemistry and Molecular Biology, Pennsylvania State University, 206 South Frear Laboratory, University Park, Pennsylvania 16802, United States

* To whom correspondence should be addressed.

Email: suzanne_walker@hms.harvard.edu

Table of contents	
contents	page
mNeonGFP DNA sequence $(5 \rightarrow 3)$	2
Figure S1. S. aureus ∆tarO is resistant to targocil and targocil-II.	3
Figure S2. Construction and map of pTarKO plasmid.	4
Figure S3. Schematic of allelic exchange with the pTarKO targeting plasmid in S. aureus.	5
Figure S4. Both <i>ArodA</i> and <i>AezrA</i> show severe growth defects at the elevated	6
temperature of 43°C	
Figure S5. UgtP is synthetically lethal with inhibition of wall teichoic acid biosynthesis.	7
Figure S6. Schematic of the two-step process to delete <i>ItaA</i> using pTarKO.	
Figure S7. Construction of an inducible <i>tarO</i> strain.	
Figure S8. Markerless deletion of spa using FRT/FLP recombinase.	
Figure S9. PCR validation of allelic exchange of target loci.	
Figure S10. Allelic exchange of the adeFGH locus with pColKO-adeFGH in A. baumannii.	
Table S1. Strains and Plasmids	13-14
Table S2. Primers	
Table S3. Synthetic lethal with <i>∆tarO</i>	

mNeonGFP DNA sequence $(5 \rightarrow 3)$

Supplementary Figures



Figure S1. *S. aureus* $\Delta tarO$ is resistant to targocil and targocil-II. (a) $\Delta tarO$, the complementation strain $\Delta tarO/ptarO$, and wildtype (wt) were tested against targocil (5, 10, or 25 µg/mL) in the presence of 0.4 µM anhydrotetracycline (aTc) to induce *ptarO* expression. The phenotype is complementable. (b) Susceptibility of $\Delta tarO, \Delta tarO/ptarO$, and wildtype were also tested against 10 µg/mL targocil-II in the presence or absence of aTc.



Figure S2. Construction and map of pTarKO plasmid. The temperature sensitive replication origin (*ori-ts*) for *S. aureus* found in the pKFC vector was removed and the *tarO* gene sequence with its native promoter was added. pTarKO contains the following features: ColE1 (replication origin for *E. coli*), Cm^R (chloramphenicol acetyltransferase), Amp^R (β -lactamase), and a multiple cloning site (MCS). Antibiotic resistant markers for pTarKO: Kan^R (aminoglycoside 3'-phosphotransferase) and tet^R (tetM, tetracycline resistance gene). Unique restriction sites are listed.



Figure S3. Schematic of allelic exchange with the pTarKO targeting plasmid in *S. aureus***.** Recombineering plasmid isolated from DH10B *E. coli* (NEB 10-beta) is irradiated with UV and electroporated into *tarO*_{off}*S. aureus*, and transformants are selected on plates containing both targocil (or targocil-II) and kanamycin. Mutants with a single crossover are screened out, and only mutants with a double crossover are selected from the double selection. A confirmed double-crossover mutant is grown in the presence of aTc to induce *tarO* for wall teichoic acid production, and the marked modified locus is transduced to other *S. aureus* strains by phage transduction. Primers for homologous arms, antibiotic marker, and confirmation of gene modification are shown as numbers: 1,AA; 2,AB; 3,BA; 4,BB; 5,CA; 6,CB; 7,KanR-F; 8,KanR-R.



Figure S4. Both $\triangle rodA$ and $\triangle ezrA$ show severe growth defects at the elevated temperature of 43°C. 5 µL of each dilution of $\triangle rodA$ RN4220 and $\triangle ezrA$ RN4220 grown to mid-log phase was spotted on TSA agar plate and incubated at 43°C or 30°C.



Figure S5. UgtP is synthetically lethal with inhibition of wall teichoic acid biosynthesis. Tunicamycin inhibits TarO, the first step of the WTA pathway in *S. aureus*, and prevents growth of a $\Delta ugtP$ mutant. Susceptibility of $\Delta ugtP$, $\Delta ugtP/pugtP$, and wildtype strains were tested against 2 µg/mL tunicamycin. The phenotype is complementable.



Figure S6. Schematic of the two-step process to delete *ItaA* **using pTarKO.** A UV-irradiated targeting plasmid was electroporated into *tarO*_{off} *S. aureus* and transformants are selected on plates containing only kanamycin to obtain mutants with a single crossover. A confirmed single crossover mutant is grown to high density and plated on both kanamycin and targocil to select for double crossover mutants.



∆ezrA, ∆tarO-P_{aTc}-*tarO*, -aTc

Figure S7. Construction of an inducible *tarO* strain. (a) Staphylococcal phage Φ 11 FRT cannot form plaques on $\Delta tarO$ cells since wall teichoic acids serve as surface receptors for phage attachment prior to infection. Labels are the same as shown in **Figure 2**. (b) In the presence of aTc, which induces *tarO* expression for WTA production, the phage generated plaques on bacterial lawns of *S. aureus*. Phage lysate was prepared from the plaques and used for phage transduction. As an example, $\Delta ezrA \Delta tarO$ -ptarO was mixed with phage Φ 11 in the presence or absence of 0.4 μ M aTc.

b



а

Figure S8. Markerless deletion of *spa* **using FRT/FLP recombinase.** (a) Schematic of the strategy to delete *spa* using pTarKO and to remove the antibiotic marker. (b) Phenotypic validation of Δspa ::FRT-Kan-FRT and Δspa ::FRT in the presence or absence of kanamycin. (c) PCR validation of allelic exchange of *spa* and removal of the kanamycin cassette.



Figure S9. PCR validation of allelic exchange of target loci. (a) Deletions of *rodA, gdpS, ltaA, mreDC, ezrA, and SAOUHSC_01649* in *S. aureus* were confirmed by PCR with primers CA and CB. **(b)** A C-terminal mNeonGFP fusion to UgtP and the exchange of the native promoter of *SAOUHSC_01050* for an aTc-inducible promoter were confirmed by PCR with primers CA and CB.

S11

b







Figure S10. Allelic exchange of the *adeFGH locus* with pColKO-*adeFGH* in *A. baumannii*. (a) A targeting plasmid isolated from *E. coli* (NEB 10-beta) is irradiated with UV and electroporated into the pass- $\Delta lpxC$ *A. baumannii* strain, and transformants are selected on colistin and apramycin. Mutants with a single crossover are screened out, and only mutants with a double crossover are selected from the double selection. To transfer the marked deletion into wildtype *A. baumannii*, a mating-out assay was performed using the $\Delta adeFGH \Delta lpxC$ strain (donor) and wildtype (recipient). (b) Mutations transferred to wildtype were confirmed by PCR using primers CA and CB.

а

Table S1. Strains and Plasmids		
Strain	Description	Source
S. aureus		
RN4220	wild type	-
HG003	wild_type	-
∆tarO RN4220	Tar	Ref. 22
$\Delta tarO_{\rm on/off}$	<i>∆tar</i> O pTP63-tarO RN4220	This study
$\Delta rodA, \Delta tarO_{on/off}$	Kan ^ĸ , Cm ^ĸ , Tar ^ĸ	This study
∆rodA RN4220	Kan ^K	This study
<i>∆rodA</i> HG003	Kan ^ĸ	This study
$\Delta ItaA, \Delta tarO_{on/off}$	Kan ^R , Cm ^R , Tar ^R	This study
<i>∆ltaA</i> RN4220	Kan ^R	This study
<i>∆ltaA</i> HG003	Kan ^R	This study
∆ltaA MW2	Kan ^R	This study
∆mreDC,∆tarO RN4220	tet ^R , Tar ^R	This study
∆mreDC RN4220	tet ^R	This study
∆mreDC HG003	tet ^R	This study
∆ezrA.∆tarO _{on/off}	Kan ^R , Cm ^R , Tar ^R	This study
∆ezrA RN4220	Kan ^R	This study
∧ezrA HG003	Kan ^ĸ	This study
AezrA USA300	Kan ^R	This study
AezrA Newman	Kan ^ĸ	This study
AndnS AtarOminet	Kan ^R . Cm ^R . Tar ^R	This study
AadnS RN4220	Kan ^R	This study
AadnSHG003	Kan ^R	This study
AgdpS USA300	Kan ^R	This study
Aspa AtarO	Kan ^R . Cm ^R . Tar ^R	This study
$A_{\text{SD2}} + HG003$	Kan ^R	This study
$A_{\text{SD2}} + H_{\text{COO3}}$	unmarked	This study
A01640 AterO RNA220	tet ^R Tar ^R	This study
$\mu \alpha t P_m N_{eon} GEP A tarO = \pi$	Kan^{R} Cm ^R Tar ^R	This study
ugtr -mNeonGFP RN4220	Kan ^R	This study
$P = -01050 \text{ Atar} \cap RN4220$	Kan^{R} Tar ^R	This study
$P_{a1c} = 0.1050 \text{ RN4220}$	Kan ^R	This study
Para-01050 HG003	Kan ^R	This study
AugtP RN4220	Kan ^R	Ref. 30
AugtP /nuatP	Kan ^R Em ^R	This study
AltaA /nltaA	Kan ^R Em ^R	This study
		The etady
A. baumannii		
ATCC19606		Ref. 43
	Kan ^R	Ref. 43
$AI_{\rm DVC}$ ATCC 19606-pass	Kan ^R	This study
Zipx0 A10010000-pass	$dapD$ (DNA CDS756: G \rightarrow A protein: none)	The olday
	WP = 0.31949781(DNA: CDS1165:insertion(A).	
	protein: frame shift)	
	baeS (DNA:CDS307:C \rightarrow T, protein: substitution: A \rightarrow T)	
ΔlpxC. ΔadeFGH ATCC19606-pass	Kan ^R , apramycin ^R	This study
AadeFGH_ATCC19606	apramycin ^R	This study
AlpxC ATCC19606-pass/plpxC	Kan ^R	This study
		· ····,
E. coli		

NEB 10-beta (DH10B™ derivative)

NEB

Continued Table S1. Strains and Plasmids		
plasmid	Description	Source
pKFC	Cm ^R , ts-Ori-replication	Ref. 5
pFLP	FLP recombinase	Ref. 15
pTM204 <i>attLint</i>	tet ^R	Ref. 15
p <i>ugtP</i>	pLOW- <i>ugtP-ItaA</i>	This study
p <i>ltaA</i>	pLOW- <i>ugtP</i>	This study
pTarKO		This study
pTP63	Ст ^к , integrative plasmid	Ref. 30
pTP63-P _{aTc} - <i>tarO</i>	Ст ^к , integrative plasmid	This study
pTarKO- <i>rodA</i> -Kan ^ĸ	rodA targeting vector	This study
pTarKO <i>-ItaA</i> - Kan ^ĸ	ItaA targeting vector	This study
pTarKO- <i>mreDC</i> -tet ^ĸ	mreDC targeting vector	This study
pTarKO- <i>spa</i> -Kan ^ĸ	spa targeting vector	This study
pTarKO- <i>ezrA</i> -Kan ^ĸ	ezrA targeting vector	This study
pTarKO- <i>gdp</i> S-Kan ^R	gdpS targeting vector	This study
pTarKO- <i>ugtP</i> -gfp-Kan ^R	ugtP targeting vector	This study
pTarKO- <i>01050-P_{aTc-}</i> Kan ^K	01050 targeting vector	This study
pTarKO- <i>01649</i> -tet ^R	01649 targeting vector	This study
pKFC- <i>rodA</i> -Kan ^R	rodA targeting vector	This study
pColKO		This study
pColKO- <i>adeFGH</i> -Apr ^R	adeFGH targeting vector	This study
pWH1266		Ref. 43
p <i>lpxC</i>	pWH1266- <i>lpxC</i>	This study

Primers Sequence (S'→ 3') restriction site in red Gene tarO-F.Kasl ATAGGCGCCCTCTGAATCGACTCATTTAATTGACCAC tarO tarO-F.Kasl ACGAGTCCCCTATTCCCTTTTACATGACTGACTACG tarO tarO-bs-telF-Kpnl ACGAGTCCCCTATTCCCTTTTACTGAGTGAATGATTACG tarO tarO-R-BcoRl ACGAGTCCCCTATTCCCTTTTCCTCTTTACGAGTGAATGATT tarO kan-F GCGAACCATTGCCGTATTCCCTTTTCTCTCTTTACGAGTA kan R tarO-A-Ba-BamHI TTACGATCATTCACCTCAAATCGCTTCAGGCGAATGATA rodA rodA-AB TTACCGATGATTCCTTATACACGCTAAAAATAGCTAC rodA rodA-BB-Sall GATTGTCGACGCAACATCCTTCTGGACGATATGCCATTAACCACATCA rodA rodA-CA GGATATGCTAAACGACGTTTATTACGACGCACATAAACTGTCCCCCCC rodA rodA-BB-Sall GATTGTCGACGCACATCATTCTGGACGATTACACTCCCCCCCC	Table S2. Primers		
tarO-F.KaslATAGGCGCGTCTGAATCGACTCCTTTAATTGACCACtarOtarO-R-EcoRIACGAGTTCCCCTTATTCCTCTTTAATGAATGACTACGtarOtarO-R-EcoRIACAGATTCCCCTATTGCCTTTATCGAATGACTGACTtarOtarO-R-EcoRIACAGATTCTTCCTTTATCCTCTTTATCGATAkan Rkan-FCCGAACCATTGCGCGTGGTGATGGAAATAATGGTTkan Rrod-A-BamHITTACGGATCGATAGAAACAATCACCGCTGArodArodA-ABCTTACCTATCACCTCAAATGGTTCGCCGCGCTGTGACGAGAATArodArodA-BBCTTACCTATCACCTCAAATGGTTCGCCGCGCTGTGACGAGATGAATArodArodA-BBTTACTGGATGGAATTGTTTTATCACCTACGGACGAATACCCCCCCC	Primers	Sequence (5` \rightarrow 3`) restriction site in red	Gene
tarO-R-EcoRIACCAATTCCCCTATTCCTCTTATGAGATGACTTACGtarOtarO-R-EcoRIACAGAATTCCTCCTTTATGAGATAAATGGTTtarOkan-FGCGAACCATTGACGATGAGATGAGTAGATGATAATGGTTkan Rrod-A-A-BamHITTAAGGATCCTTTCAGCCGCATACGGTCAAkan Rrod-A-BCTTACGGATGAAAGAATGACCCCGCGCGTCTTAGAGAGATAArodArod-A-BaTTACGGATGGAATAGTGTTCAGGCCGCGCTCTGACGAGATGAATArodArod-A-BaTTACGGATGGAATGGTTTAGGTCCTAGCGCGCGTCTGACGAGATGATArodArodA-BBGATGTCGACGCACATCCTTCGACGAATATGTCGATTTATACCATCrodArodA-BB-SallGATGTCGCACGCACATCCATCTGCACCAATATAArodArodA-CBAGATAGTCAAAGGGTTTATATACAGCGCCltaArad-A-CBAGTATAGTAAGGATCTAAAAGGGTTTATATACCACCCGCltaAltaA-ABATTACTGCACGAATGCAAATGGTCGCGCGCGCGATATACATCCCTCGCCAANTCltaAltaA-ABATTACTGCACAAAGGGTTTTATACCAGCCGCCltaAltaA-BATTACGGATGCAATGCTTAAGCGTACCTAGCGTCGCGCGltaAltaA-BATTACTGCACAACAATGGTAGCCTGACGCGCCCCCGATltaAltaA-CBCATGTCGCACAACCAATGGTTAAGCGTAGCCGCGCATATACATCCCTCGCGCAltaAltaA-CBAAGAGTATTAGATAGATGAATGATGTTAAGCGTCGCGGATATACATGCTCCGGCGltaAgdpS-ABATTGCGACGATTGATTTATGltaAgdpS-ABATTGCGACGATGATTGTTTAGGTCGCAATAATGGTCGgdpSgdpS-BBTTACGGATGAATGATGTTTAAGCGCGAAAAATGATGCTGCgdpSgdpS-CBTTGCGACCACATCGATGATGGTTGGCAAAGAAGCCCCATATAgdpSgdpS-CBTTGCGACGCACGATGATGGTTGGCGAAGAAGCCCCTTATAGGgdpSgdpS-CBTTACGTATGCACCAAGGAGATAGCTGCGCATAGCGGTAGGTA	tarO-F-KasI	ATA <mark>GGCGCC</mark> GTCTGAATCGACTCCTTTAATTGACCAC	tar0
tartO-hs-tetF-Kpn1AGAGGTACCATATCGATGAAGGTGAATAAATGGTTtarOtarO-R-EcoRIACAGATTCTTCATTTCGATTCCCTTTATGAGATtarOkan-FCCGAACCATTTCAGGTGATAGGTAAGATTATkan Rkan-RTCCTAGGTACTAAAAGAATTCATCCCGTTCATGAGGAArodArodA-ABTTACGGATGACTTCATCACCGTCAGGCGACGATGAGATArodArodA-ABCTTACCTATCACCTCAAATGGTTGGCGGCGCTGTTGAGGAGATGATArodArodA-BATTACGGATGGAATTGTTTTAGTACCTAGGCGACGATATGTGGATTATACCATCrodArodA-BAGATTGTCGACGCACATCATTCGACGACAAGGAGTAArodArodA-BAGATTGTCGACGCACATCATTCGACACAAGGAGTAArodArodA-CAGGATAGTCGATTGTCTGAAACGGCTCArodAItaA-AA-BamHITTAGGGATGCTATCCTACAAGGGTTTAATCAAGGCGCCItaAItaA-ABTTACTGGATGACATTGTTTTATCACCGGGATATACATGCCTCGCGAAATTCCTItaAItaA-BBTTACTGGATGACAATGCTTTAACCAAGGCGTCCItaAItaA-BBTTACTGGATGACAATGCTTTAACCAAGCGGCCGATItaAItaA-CBAAAAGTATTAGCTATCACCTGCAAGGTGTCGCGCGATItaAItaA-CBAAAAGTATTAGATAAGCTAAATCAATGTCCItaAItaA-CBAAAAGTATAGATAAGCTAAATCAATGTCCItaAItaA-CBATTGTCGACACACAATTGGTTAGCCCACATACgdpSgdpS-BATTCTGCATCACCCTAATACGATGCTItaAItaA-CBATACCAATTAGCTATAGATAGGTCGCCCCCATTACgdpSgdpS-BATTCTGCAGCACACACATGCTGCTCTTAgdpSgdpS-CBTTGCGACACACATGCTGCTGCTGCATAAATGATCCCGGGCAAACACACAC	tarO-R-EcoRI	AC <mark>GAATTC</mark> CCCTATTCCTCTTTATGAGATGACTTACG	tar0
tarO-R-EcoRIACAGAATTCTTTCATTCCCTATTCCTCTTATCAGATtarOkan-FCCGAACCATTTGAGGTAATAGGTAAGATTATkan RrodA-AB-BamHITTAAGGATCCTTCATAACAGCTAACGCGTAArodArodA-ABCTTACCTAACACCTCAATGGTCGCCGCGTGTGACGACGAATGACCATCrodArodA-BBCTTACCTAACGCTCCAAATGGTTCGCCGCGGTGTGACGACGAATGACCATCrodArodA-BB-SallGATTGTCGACGCACACTATTCTGAACGAATGACTCACTCrodArodA-CBAGTATGCTAAGCACTCAAATGATCAACGACTCArodArodA-CBAGTATGCTAAGGAATGTAAATGAACGAGTGArodArodA-CBAGTATGCTAAGGAATGTAAATGAAGGAGTGArodArodA-CBAGTATGCTAAGCAATGCTAACGACGCCItaAItaA-ABTTACTGGATGCAATGCTAACGCGCCItaAItaA-BATTACTGGATGAATTGTTTTTAGTACCCGCGCItaAItaA-BATTACTGGATGAATTGTTTTTAGTACCCGCGATTAAACATGCTACGCGCCItaAItaA-BATTACTGGATGCAACACAAATGGTAAGCCTACGCGACTATAACATGCTCGCGAATTCItaAItaA-CBAAAAGTTATGCTAACAATGGTAAGCCTACGCGCCCCATTACgdpSgdpS-ABATGTTGCGACATCCAATGAATGCATGGCCItaAItaA-CBATTGTCGCACATCCAATCAATGCAAGCCCCCCCTATCgdpSgdpS-CAAATAGCAATTATGCTTAAGGAGCCTATAATGCATGCCGCGCAAACAAA	tarO-rbs-tetF-KpnI	AGA <mark>GGTACC</mark> ATATCGATGAAGGTGAATAAATGGTT	tar0
kan-FCCCAACCATTTGAGGTCATAGGTAGGTAGAGATTATkan Rkan-RTCCAAGGTACTTAGGTCAAAAACAATTCATCCAGTAAkan RrodA-AA-BamHITTAAGGATCCTTCTATAACAAGCCTACGGTCAArodArodA-ABCTTACCTATCACCTCAAATGGTTGCGCGCGCTGTTGACGAGATGAATArodArodA-BBCTTACCTATCACCTCCAAATGGTTGCCGCGCTGTTGACGAGATGACATArodArodA-BB-SallGATTGTCCAACCACCCACACATTCTGAACAATAAArodArodA-BB-SallGATATGTCCACACCACACATTCTGAAACACGCTCCrodArodA-CAGGATAGTCATTCCTAAAACTGGTACTCCTrodAItaA-ABATAATCTACCTCAAAATGGTTGCTGCGGGCGATATCATGCCCTCTCGCAAATTCItaAItaA-ABTTACTGGATGGATTGTTTTAGGTCCCGGGATTACATGCCTCTCGCGAAATTCItaAItaA-BBCATTGTCGCACACACAATGGTTGCGCGGCGATATACATGCCTCTCGCGAATTCCTGCGGCGItaAItaA-CBATACTTACCTATCACCTCAAATGGTTGCGCGGCGATATCATGCGCGCATATACAGGGGGAAACAAGGGGGAAACAAGGGGGAAACAAGGGGGAAACAAGGGGGAAACAAGGGGGAAACAAGGGGGAAACAAAGGTGTGTGGCGACACAAGGCGCCCTATCgdpSgdpS-ABATGCTGCGACTCCCAATGGTTGCGCACTATCgdpSgdpS-CAATACCAAGTTAGTTAGGTGCGCCACTATCgdpSgdpS-CBTTGCAAATGCAATGATCGCTGCTGCTAACGCTCCTTATGgdpSgdpS-CBTTGCAAATGCAAGCATCATTAGGTTGGCCACCTCTATCgdpSgdpS-CBTTGCAAATGCAAGAATGATCAGGCTGCTCTCTAgdpSgdpS-CBTTGCAAATGCATGATGGTGGTGGCAACACCTCTATAGGGGGAAACAAAGTAgdpSgdpS-CBTTGCCAATGCCGAACAGCACCTCTCTACgdpSgdpS-CBTTGCCAATGCCGAAGAAGACCCCCTATGGTGGAGGTATAGGTGGAAGGTAAgdpSgdpS-CBTTGCCAATGCCGACATAGGTGCTGCTAAGGTGGATAGGTGGTAGGTA	tarO-R-EcoRI	ACA <mark>GAATTC</mark> TTTCATTCCCTATTCCTCTTTATGAGAT	tar0
kan-RTCCTAGGTACTAAAACATTCATCACAGCTAAkan RrodA-AB-BamHITTAAGGATCCTTCTATAACAAGCTTACGCGTCACAGAATArodArodA-ABCTTACCTATCACCTCAAATGGTTCGCCGCCTGTGACGAGTGAATArodArodA-BACTTACCTATCACCTCAAATGGTTCGCCGCCTGTGACGAGTGAATArodArodA-BB-SallGATTGTCGACGCCACATCATTCGAACAATAArodArodA-CAGGATAGTCATTGCTAAGCGAACGATCATCCrodArodA-CAGATATGCTAAGCGAATGAATGCAACGGCCCrodAItaA-AB-BamHITTAAGCGACCTACAAAGCGTTTATTATCACCGCGCItaAItaA-ABATAATCTTACCTATCACCTCAAAAGCGTCGCGCCItaAItaA-BATTACTGGATGAATTGCTTTAGTACGCGGCGCCATAACATGCTTCGCGCAATTCItaAItaA-BATTACTGGACAACAAATGCTTAGCGCTCCGCGCATItaAItaA-BATTACTGGACAACAAATGCTAAGCGTAAGCGACTCAAATACATTGCTCCGCGAAATACATGCCCItaAItaA-BATTACTGGACAACAAATGCTAAGCGTAAGCGATTAAACAATGCATTGCCItaAItaA-CACGTCATTGAGCACGCATTTATTATGItaAItaA-CACGTCATTGAGCACGCATTTAATAGCAATGCTCCCCCAATAACAGCAAAGCAAgdpSgdpS-ABATTGTCGACACCCAAATGCATTAACGCAATGCCTgdpSgdpS-ABATTGTCGACACCCAAACGCTTAATAGCATGCCCCCCCCCC	kan-F	GCGAACCATTTGAGGTGATAGGTAAGATTAT	kan R
rodA-AA-BamHITTAAGGATCCTTCTATAAGAACCTTACGGTCAArodArodA-ABCTTACCTATCACCTCAAATGGTTGGCGGGCGTTGAGGAGAGATArodArodA-BATTACTGGATGAATTGTTTAGTACCTAGGAGGATATGTCGATTATACCATCrodArodA-BB-SallGATTGTCGACGACACTATTCTGAACAAATAAArodArodA-CAGGATAGTCATTCCTAAAACTGCTACTTCrodArodA-CBAGTATGTCATTCCTAAAACTGCTACTTCrodArodA-CBAGTATGTCATCACAGGAATGTAATGCAGGAGTGArodAItaA-ABATACTTACCTACAAAGGGCGCATTACATGCCGCACATACAT	kan-R	TCCTAGGTACTAAAACAATTCATCCAGTAA	kan R
rodA-ABCTTACCTATCACCTCAATGGTTCGCCGCGTGTTGGCGAGATGATArodArodA-BATTACTGGATGAATTGTTTTAGTACTTGGACGATATGTCGATTTATACCATCrodArodA-BB-SallGATTGTCGACGACATCATTTCTGACAAATAAArodArodA-CAGGATTGTCGACGCACATCATTCTGAACAATAAArodArodA-CAGGATTGTCGACGACATCATTCGACAAATAAArodArodA-CBACTATAGTAAGGAGCATTTAATCAACGCATCArodArodA-CBACTATAGTAAGGAAGCATTCAAATCAACGACTCArodAltaA-AB-BamHITTAAGGATCCTACAAAGGGTTTTATTACACGCCGCCltaAltaA-ABATAATCTTACCTATCACCTCCAATGCTTTCACACGCCTCTCGCAAATTCltaAltaA-BBCATTGTCGACAACAATGGTTACCTAGGATTTAAACAATACATTTTATTCTGGGCAltaAltaA-CBCGTCATTGGACAACAATGGTAAGCTGACGCGCCGATltaAltaA-CBCGTCATTGGACAACAATGGTAAATCAATGGTCCltaAltaA-CBATTGTCGACACACCAATTAATAGCTACATGTCgdpSgdpS-AA-SallATTGTCGACATCCAATCATTAAACCACTCCTTCCTTAgdpSgdpS-CBTTACGGATGAATTGGTTTACCACTCCAGGACAAAAAGATCAAGGGCGAAACAAAGTAAgdpSgdpS-CBTTGACGATCCATCACGCTATTCCTCCTTAgdpSgdpS-CBTTGAAAATGATAAGCATTAAGCTTGCCTACCACGCCCATATAgdpSgdpS-CBTTGAAAATGATAGGAAAAGTACTTAGCGCCAACCACCTCTATTAGgdpSmreDC-AB-BamHITTAACGATCCATCAGCCATACCCCACACACCCTCTATTAGgdpSmreDC-BATACGTCCAAACCCCCAATTAGCCCCCCAATAGTGGAGGTATAmreDCmreDC-BATTGATGCAAACCACGCCCTATATCCCCCCAAACGCGATGACGCATAspaspa-SABTACTCTTGCGAAACCACGCCCTATATCCCCCCCAATAGCGCATAspaspa-ABTACCTTACCCTCAAAGCTCTCCAAACGCGTTAGCACCCCTTATTGTAACACACAC	rodA-AA-BamHI	TTAA <mark>GGATCC</mark> TTCTATAACAAGCTTACGGTCAA	rodA
rodA-BATTACTGGATGAATTGTTTTAGTACCTAGGACGATATGTCGATTTATACCATCrodArodA-BB-SallGATTGTGCGACGCACATCATTTCGAACAAATAAArodArodA-CBAGTATGTCAGCACGACATTCATTCGAACAAATGAArodAltaA-CBAGTATGTCAGCACGACATTCATATGAGCGGGTArodAltaA-ABTTACTGGATGACATTGTTATACAGCGCGCltaAltaA-BAATAATCTTACCTATCACACTGCACATGGCGGATTAAAAACAATCAGTTTTATTTCTCGGGAltaAltaA-BATTACTGGACAACAATGGTAGGCGGCGATltaAltaA-BB-SallGATTGTCGACAACAATGGTAGGCGGCGATltaAltaA-BB-SallGATTGTCGACAACAATGGTAGCGCGCGATltaAltaA-CBAAAAAGTATTAGATAAAGCCTCAAATGGTCGltaAltaA-CBAAAAAGTATTAGATAAAGCCTCAATGTCCgdpSgdpS-AA-SallATTGTGGACATTGCACATCATGGTCGGAATAAATGAATCAAGGGCGGAAACAAAGTAAgdpSgdpS-BB-BamHICCGCAAGCTTAAATGAACGCTCACTCTCTTAgdpSgdpS-CBTTACTGGATGAATTGTTGACACAGCTATGCTgdpSgdpS-CBTTACGAATCATAGCAGCATGCTCTCTTAgdpSgdpS-CBTTACGAACCATACACCACCCCACAGCACGCCAGAACAAAGTAAgdpSgdpS-CBTTACGAATCATAGCAGAAAGTCATGCTTGATAmreDCmreDC-ABTTACGTACACCCAACACTGCCACAGAGCACTCCTATATGGmreDCmreDC-BATTACGTACACCCCAACATGGTCGCCACCCAGAACCTCCATATAGGmreDCmreDC-BATACGTATCACCCTCAAATGGTCGCCACCCAGAACCTCCATATAGGCATAspamreDC-CACAAGCCAAAATCAGCACACCACACCCTACATTAGCACACACCACACCCCCAATAGCTACCATCspamreDC-CACAAGCTAAAACCATTGGCAATAAGGAACTTCATTATACCCCCTCATATAGGCACAACCAATCACACACCCCCCAAAGCATAGGAATAGGAATCACTTTTAGAGCAAACCAATCACACACA	rodA-AB	CTTACCTATCACCTCAAATGGTTCGCCGGCTGTTGACGAGATGAATA	rodA
rodA-BB-SallGATTGTCGACGCACATCATTCTGCACACAATAAArodArodA-CAGGATAGTCATTCCTAAAACTGCTACTTCrodArodA-CBAGTAGTCAGTAAGCAATGTAAACTGACGCACTCArodAltaA-AA-BamHITTAAGGATCTACCAAGAGGTTTTATTACAGCGCCCltaAltaA-ABATAATCTTACCTACACAAGGGTTTAATACATGCCTCCGCAAATTCltaAltaA-BATTACGGATGATGATTGTTTAGTACCTAGGATTTAAACATACAT	rodA-BA	TTACTGGATGAATTGTTTTAGTACCTAGGACGATATGTCGATTTATACCATC	rodA
rodA-CAGGATAGTCATTTCCTAAAACTGCTACTTCrodArodA-CBACTATAGTAAGAATGTAAAGAAGGACTGArodAItaA-AA-BamHITTAAGGATCCTACAAAGGGTTTATTACAGCCGCCItaAItaA-ABATAATCTTACCTATCACCTCAAATGGTTCGCGGATATACATGCCTCTGCGAAATTCItaAItaA-BAATAATCTTACCGATGAATTGTTTATTACAGCCAGCACTItaAItaA-BATTACTGGATGAATTGTTTTATGTACCTAGGATTTAAACAATACATTTATTT	rodA-BB-SalI	GATT <mark>GTCGAC</mark> GCACATCATTCTGAACAAATAAA	rodA
rodA-CBACTATACTAACGAATCTAAATGAAGGACTCArodAItaA-AA-BamHITTAAGGATCCTACAAAGGGTTTATACAGCCGCCItaAItaA-ABATATCTTACCTATCACCTCCAAATGGTTCGCGGATATACATGCCCTCTGCGAAATCCItaAItaA-BATTACTGGATGAATTGTTTAGTACCTAGGATTAAAACAATACATTTTATTTCTCGGCAItaAItaA-BB-SallGATTGTCGACAACAAATGGTAGCGTGCCGATItaAItaA-CBAAAAGTATTAGATAAGCTAATCAATGTGCItaAItaA-CBAAAAGTATTAGATAAGCTAAATGAATGATCCCCCCCTATCgdpSgdpS-AA-SallATTCTCGACATCCAATAGTATGCTTCGACATAAATGAATCCAAGATCAAAGTAAgdpSgdpS-ABATTCTTACCTATACATGCATCCAATAGTAGCTCCACCAAACAAA	rodA-CA	GGATAGTCATTTCCTAAAACTGCTACTTC	rodA
ItaA-AA-BamHITTAAGGATCCTACAAAGGGTTTATTACAGCCGCCItaAItaA-ABATAATCTTACCTATCACCTCAAATGGTTCGCGGATATACATGCCTCTCGCAAATTCItaAItaA-BATTACTGGATCAACTTAGTACTCACGGAGATTAAACAATACATTCTTTATTTCTCGGCAItaAItaA-BB-SallGATTGTCGACACAAATGGTAAGCGTGCCGATItaAItaA-CACGTCATTGAGCACAGATTTATTATGItaAItaA-CBAAAAGTATTAGATAAGCTAAATGATGCCgdpSgdpS-AA-SallATCTTACCTATCACCTCCAAATGGTCGCAATAAATGAATCCAGGGGAAACAAAC	rodA-CB	AGTATAGTAAGGAATGTAAATGAAGGAGTGA	rodA
ItaA-ABATAATCTTACCTATCACCTCAAATGGTTCGCGGATATACATGCCTCTGCAAATTCItaAItaA-BATTACTGGATGAATTGTTTTAGTACCTAGGATTTAAACAATGCATTCTTATTTCTCCGGGAItaAItaA-BB-SallGATTGTCGACAACAAATGGTAAGCGTGACGGATItaAItaA-CBAAAAAGTATTAGATAAGGTAAATCGATGGCItaAdqbS-AA-SallATTGTCGACATCCAATCATTATTATGItaAdqbS-AA-SallATTGTCGACATCCAATCATTAAAGGTCAATCGATGGCItaAgdpS-ABATTCTTACCTATCACCTCAATGGTTCGCGATAAATGGATTCCAGGGCGAACAAAGTAAgdpSgdpS-BATTACTGGATGAATTGTTTTAGTACCTAGGAAAAATGAATCAAGGGCGAAACAAAGTAAgdpSgdpS-BATTACTGGATGAATTGTTTTAGTACCTAGGAAAAATCAAGGGCGAAACAAAGTAAgdpSgdpS-CAAATACAAATTATCCCATACAGCTATGCTgdpSgdpS-CBTTGAAAATGATAGAGCATAGGCTGAAGACTmreDCmreDC-AA-BamHITTAAGGATCCATCAAGGTTAGGCGGAAGAACCTCTATTATGmreDCmreDC-AA-BamHITTAAGGATCCATCAATGATTAGGATGCCGCACCAGAAACCTCTATTATGmreDCmreDC-BATTACCTATCACCCTCAATGGTTGGCCACCAGAAACCTCTATTATGmreDCmreDC-BBGATTGTCGACACCACGACCTCCATTAGGTGAGGAGACTmreDCmreDC-CACAATGCTACAAACCTCCATAAAGGAACTCCATTAGTGAGTAGCTAAmreDCmreDC-CBCGGTGCATTTAGGAAATTAGGACTCCATCAATGCTmreDCmreDC-CBCGGTGCATTTAGGAACTTCATAGACGGATTAGTACGGATTAGCAGCATAspaspa-ABTACTCTCAGGAAATGGAACTTCATTAACCCCCTGTATGTA	ltaA-AA-BamHI	TTAA <mark>GGATCC</mark> TACAAAGGGTTTTATTACAGCCGCC	ltaA
ItaA-BATTACTGGATGAATTGTTTAGTACCTAGGATTTAAACAATACATTTATTT	ltaA-AB	ATAATCTTACCTATCACCTCAAATGGTTCGCGGATATACATGCCTCTCGCAAATTC	ltaA
ItaA-BB-SallGATTGTCGACAACAAATGGTAAGCGTGCCGATItaAItaA-CACGTCATTGAGCACGATTTATTTATGItaAItaA-CBAAAAAGTATTAGATAAGCTAAATCAATGGTCGItaAgdpS-AA-SallATTGTCGCACATCCAATCAATTATATAACAGTGCCCACTATCgdpSgdpS-ABATCTTACCTATCACCTCAAATGGTTCGCAATAAATAGATTCCAGGCGAAACAAAGTAAgdpSgdpS-BB-BamHICGCGAAGCTTAATAGCATGCTTTAACAGTCCTCGCTATAACAGGGCGAAACAAAGTAAgdpSgdpS-CBTTGACAATGATAGCATGCTTTAACAGTCCTTCCTTAgdpSgdpS-CAAATACAAATTATCCCATACAGCTATGCTgdpSgdpS-CATTAAGGATCCATCAGGATAGCTGTGAAGACTmreDCmreDC-AA-BamHITTAAGGATCCATCAGGATAGCTGTGAGAGCTmreDCmreDC-AA-BamHITTAGCGATCACTGATGGTTGGCACCCGAGAACACTCTATTATGmreDCmreDC-BBTATGTTCAATAAATAGCTTGAGAGAGCACACCTCTATTATGmreDCmreDC-BB-SallGATTGTCGACACCACGAGCACACACTGCAAGACGCTGTATATGmreDCmreDC-BB-SallGATGCTGCACACCAGGACTCCAATAGCmreDCmreDC-CBCCGTCGGATTAGCTCGCAATATGCmreDCmreDC-CBCCGGGGATCCAGATTATGGTAGCACACTTAGTAGTAGAGATAGCATTspaspa-ABTATCTCTCAGAAAGTATAGGAACTTCATTAATACGGATTTAGTACAGCATAspaspa-ABTATTCTCTAGAGAATAGGAACTTCATTAATACGGATTTAGTACAGCATAGATATCATTTATTT	ltaA-BA	TTACTGGATGAATTGTTTTAGTACCTAGGATTTAAACAATACATTTTATTTCTCGGCA	ltaA
ItaA-CACGTCATTGAGCACGATTTATTATGItaAItaA-CBAAAAAGTATTAGATAAGCTAAATCAATGACCItaAgdpS-AA-SallATTGTCGACATCCAATCATTAAAGCCCACATATCgdpSgdpS-ABATCTTACCTATCACCTCCAATGTTCGCAATAAATAGATTCCAGCGACTATAACAGAgdpSgdpS-BBMACTCTAACCTACACATCATTGTTTAGCATCAGCAAAAAATCAAGGGCGAAACAAAGTAAgdpSgdpS-CAATACCAAATGATTGTTTACAGTCATCACGCATCTCTCTAgdpSgdpS-CBTTGAAAATGATAAGCATGCTTTACCAGTCATGCTgdpSgdpS-CBTTGAAAATGATAGAGAAAAGTACTGTTGGATAgdpSmreDC-AA-BamHITTAACGATCCATCACTGATAGGCTGCCACGCAGAACACCTCTATTATGmreDCmreDC-ABTATGTCGACACCACGAGCTACAGTTAGGCTGCGCAGCAGCTAmreDCmreDC-BBGATTGTCGACACCACGAGCCTACATTTCmreDCmreDC-BB-SallGATTGTCGACACCACGAGCCTACATTTCCmreDCmreDC-CACCGAGCGATTTAGCGAATAAGTmreDCmreDC-CACCGTCGATTTAGCGAATATGCmreDCmreDC-CACCGTCGATTTAGCGAATAAGTspamreDC-CACCAGCGAATTTAGCAATATATCTTACTATCTACTGCCAACCACGACTAspaspa-ABTACTTTCTAGAGAATAGGAACTTCAATAAGCGATTAGGACTAGCATAspaspa-ABTATTCTCTAGAGAATGGAACTTCAATAATCTTAGTACAGCATAspaspa-ABTATTCTCTAGGAAATAGGAACTTCAAAACAAACAATACACAACGAATACATTTATTT	ltaA-BB-SalI	GATT <mark>GTCGAC</mark> AACAAATGGTAAGCGTGCCGAT	ltaA
ItaA-CBAAAAAGTATTAGATAAGCTAAATCAATGTGCItaAgdpS-AA-SallATCTTACCACATCCAATAGCTAATAAAGCCCCACTATCgdpSgdpS-ABATCTTACCTATCACCTCAAATGGTTCGCAATAGATAGATCCAGCGACTATAACAGAgdpSgdpS-BATTACTCGATGAATTGTTTTAGTACCTAGGAAAAAATCAAGGGCGAAACAAAGTAAgdpSgdpS-BB-BamHICCGAAGCTTAATAGCATGCTTTAACAGTCCTTCCTTAgdpSgdpS-CAAATACAAATTATCCCATACAGGTAATGCTgdpSgdpS-CBTTGAAAATGAAATGAGGAACATGTGTGATAgdpSmreDC-AA-BamHITTACCTATCACCTCAAATGGTTGCCCACCCAGAACACCTCTATTATGmreDCmreDC-ABTTACTGTCAACACCACAATGGTTGCGCACCGAGAACACCTCTATTATGmreDCmreDC-BBCGATGTCACACACCACGACCTCCATATGGTGGATGGTAmreDCmreDC-BB-SallGATTGTCGACACCACGACCTCCTAATAGGTmreDCmreDC-CBCCGTCGATTTAGGAATAAGGmreDCmreDC-CBCCGTCGACTTTAGGAATATGGmreDCmreDC-CBCCGTCGACTTTAGGAACTCCTTAATACGCAspaspa-ABTACTTTCTGAGAAATGGACTTCAATAACTGGATTTAGTAACGCATAGspaspa-ABTACTTTCTAGAGAATAGGAACTTCAATAACCGATATGGATATTGTAAAGTCATCspaspa-BB-SallTGCAGGCCGCACTCAGATTTAGGACCTCCAATTATGTAACGCAATGGATATGATTTATTACCspaspa-BB-SallTGCAGGCCGAACCAATGGGAGGACTTCCAACAACAACAACAACAACAACGATAGATA	ltaA-CA	CGTCATTGAGCACGATTTATTATG	ltaA
gdpS-AA-SallATTGTCGACATCCAATCATTAAAGCCCCACTATCgdpSgdpS-ABATCTTACCTACCTCAACTCGACTGGGTCGCAATAAATAGATTCCAGCGACTATAACAGAgdpSgdpS-BATTACTGGATGAATGTTTTAGTACCTAGGAAAAAATCAAGGGCGAAACAAAGTAAgdpSgdpS-BATTACTGGATGAATTGTTTAACACTCAGGAAAAAATCAAGGGCGAAACAAAGTAAgdpSgdpS-CAAATACAAATTATCCCATACGCTTTACACGTCTTCCTTAgdpSgdpS-CBTTGAAAATGATAGAAGAAAAGTACTGTTGGATAgdpSmreDC-AA-BamHITTAAGGATCCATCATCATGATTAAGGCTGAACACCTCTATTATGmreDCmreDC-ABTATGTTCCAATAAAGTATGGTTGGCCACCCAGAACACCTCTATTATGmreDCmreDC-BBGATTGTCGACACACCACGACCCACAGATGGTGGAAGACTTAmreDCmreDC-CBCCGTGGATTTTAGCGAATAATGmreDCmreDC-CBCCGTCGATTTAGCGAATAATGmreDCmreDC-CBCCGTCGATTTTAGCGAATAATGmreDCmreDC-CBCCGTGGATTTTAGCGAATAAGTTGAGAspaspa-ABTACTTTCTAGGAAATAGCTTCATAAAATACTTAGAspaspa-ABTACTTTCTAGGAAATAGGAACTTCATTAATACCCCCCTATGTATTGTAAAGTCACCspaspa-BATACTTTCTAGGAAATAGGAACTTCATTAATACCCCCCGTATGTAT	ltaA-CB	AAAAAGTATTAGATAAGCTAAATCAATGTGC	ltaA
gdpS-ABATCTTACCTATCACCTCAAATGGTTCGCAATAATAGATTCCAGCGACTATAACAGAgdpSgdpS-BATTACTGGATGAATTGTTTTAGTACCTAGGAAAAAATCAAGGGGCGAAACAAAGTAAgdpSgdpS-BACCGAAGCTTAATAGCATGCTTTAACAGTCCTTCCTTAgdpSgdpS-CAAATACAAATTATCCCATACAGTCCTTCGTTGATAgdpSgdpS-CBTTGAAAATGATAGCCATACAGTCTTGATAgdpSmreDC-AA-BamHITTAAGGATCCATCATGATTAAGGCTGAAGACTmreDCmreDC-ABTTACCTATCACCTCAAATGGTTCGCCACCCAGAACACCTCTATTATGmreDCmreDC-BATATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGCTATAmreDCmreDC-BB-SallGATTGTCGACACCACAGACCTCCAATAGCTmreDCmreDC-CBCCGTCGATTTAGGGAATAATCmreDCmreDC-CBCCGTCGATTTAGGGAACATTTGGGmreDCmreDC-CBCCGTCGATTTAGGAACATTTGGGmreDCmreDC-CBCCGTCGATTTAGGGAATAATCmreDCmreDC-CBCCGTCGATTTAGGAACATTTGGGmreDCtetM-FCTAAGCTTAATTGGAACATTATCTTACTTtetRspa-ABTACTTTCTCAGAAAGTATGGAACTTCAACAACAATCGGATTTAGTAAGCAATAspaspa-ABTACTTTCTCAGAAAGTATAGGAACTTCAACAAACAAAACAAAACAAAGAATACAATGATATCATTTATCCspaspa-BATATCTTCTCAGAAAGTATAGGAACTTCAAAAAACAATACAAACAA	gdpS-AA-Sall	ATT <mark>GTCGAC</mark> ATCCAATCATTAAAGCCCCACTATC	<i>adpS</i>
gdpS-BATTACTGGATGAATTGTTTAGTACCTAGGAAAAAATCAAGGGGGAAACAAAGTAAgdpSgdpS-BB-BamHICCGAAGCTTAATAGCATGCTTTAACAGTCCTTCCTTAgdpSgdpS-CAAATACAAATTATCCCATACAGCTATGCTgdpSgdpS-CBTTGAAAATGATAGAAGAAAAGTACTGTTGATAgdpSmreDC-AA-BamHITTAAGGATCCATCATGATTAAGGCTGAACAGCTmreDCmreDC-ABTTACCTATCACCCTCAAATGGTTCGCCACCCAGAACACCTCTATTATGmreDCmreDC-BB-SallGATTGTCGACACCAGGACCTACTTTTCmreDCmreDC-CBCGCGCGATTCTAGCAACACCCCCAGAACACCTCTATTATGmreDCmreDC-CBCGCTCGATTTTAGCGAATAGGmreDCmreDC-CBCCGTCGATTTTAGCGAATAGGmreDCmreDC-CBCCGTCGATTTTAGCGAATAGGmreDCtetM-FCAACCCAAATCTCGCAATTGGAmreDCtetM-FCAACCCAAATCTCGCAATTAGGAmreDCtetM-RCTAAGTTATTTTATGGAACATATATCTTACTTtetRspa-AA-BamHICCCGGGGATCCACATTTAGGAACTTCAATAACCCCCTGTATGTA	gdpS-AB	ATCTTACCTATCACCTCAAATGGTTCGCAATAAATAGATTCCAGCGACTATAACAGA	advS
gdpS-BB-BamHICCGAAGCTTAATAGCATGCTTTAACAGTCCTTCCTTAgdpSgdpS-CAAATACAAATTATCCCATACAGCTATGCTgdpSgdpS-CBTTGAAAATGATAGAGAAAAGTACTGTTGATAgdpSmreDC-AA-BamHITTAAGGATCCATCATGATTAAGGCTGAAGACTmreDCmreDC-BATATACTTACACCTCAAATGGTTCGCCACCCAGAACACCTCTATTATGmreDCmreDC-BATATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGTAAmreDCmreDC-BBGATTGTCGACACCACGACCTACATTTCmreDCmreDC-CBCGTGCGATTTAGCGACACCACGACCTACATTTCmreDCmreDC-CBCGTCGATTTAGCGAATAATGmreDCmreDC-CBCCGTCGATTTAGCACACCACGACTACATTGTACTTtetRtetM-FCAACCCAAATCTCGCAATTATCTTACTTtetRspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATCGCATTAGTACTAGACATACCTCspaspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCTGTATGTA	gdpS-BA	TTACTGGATGAATTGTTTTAGTACCTAGGAAAAAATCAAGGGCGAAACAAAGTAA	adpS
gdpS-CAAATACAAATTATCCCATACAGCTATGCTgdpSgdpS-CBTTGAAAATGATAGAGAAAAGTACTGTTGATAgdpSmreDC-AA-BamHITTAAGGATCCATCATGATTAAGGCTGAAGACTmreDCmreDC-ABTTACCTATCACCCCACAATGGTTCGCCACCCAGAACACCTCTATTATGmreDCmreDC-BATATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGGTAG	gdpS-BB-BamHI	CCGAAGCTTAATAGCATGCTTTAACAGTCCTTCCTTA	advS
GetP 1 FromOffgdpS-CBTTGAAAATGATAGAGAAAAGTACTGTTGATAgdpSmreDC-AA-BamHITTAAGGATCCATCATGATTAAGGCTGAAGACTmreDCmreDC-ABTTACCTATCACCTCCAAATGGTTCGCCACCCAGAACACCTCTATTATGmreDCmreDC-BATATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGTTAmreDCmreDC-BB-SallGATTGTCGACACCACGACCACACATTTCmreDCmreDC-CACAATGCTACAAACCTCATATAGGmreDCmreDC-CBCCGTCGATTTAGCGAATAATGmreDCmreDC-CBCCGTCGATTTTAGCGAATAATGmreDCtetM-FCAACCCAAATCTCGCAATTGAGtetRtetM-FCAACCCAAATCTCGCAATTAGTATATCTTACTTtetRspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCTGTATGTA	gdpS-CA	AATACAAATTATCCCATACAGCTATGCT	adpS
Bit PDC-AA-BamHITTAAGGATCCATCATGATTAAGGCTGAAGACTmreDCmreDC-ABTTACCTATCACCTCAAATGGTTCGCCACCCAGAACACCTCTATTATGmreDCmreDC-BATATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGTTAmreDCmreDC-BB-SallGATTGTCGACACCACGACCTACATTTCmreDCmreDC-CACAATGCTACAAACCTCCTAATACGCmreDCmreDC-CBCCGTCGATTTAGCGAATATGmreDCtetM-FCAACCCAAATCTCGCAATTTGAGtetRtetM-RCTAAGTTATTTTATGAACATATCTTACTTtetRspa-ABTACTTTCTAGAGAATAAGGAACTTCATTAAACCAATCGGATTTAGTACAGCATAspaspa-ABTACTTTCTAGAGAATAGGAACTTCATAAACAAACCAATACCACAACGATAGCATATCATTTTATCspaspa-BATATTCTCTAGAAAGTATAGGAACTTCAAACAAACAATACACAAACGATAGATA	gdpS-CB	TTGAAAATGATAGAGAAAAGTACTGTTGATA	adpS
mreDC-ABTTACCTATCACCTCAAATGGTTCGCCACCAGAACACCTCTATTATGmreDCmreDC-BATATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGTTAmreDCmreDC-BB-SallGATTGTCGACACCACGACCTACATTTCmreDCmreDC-CACAATGCTACAAACCTCCTAATACGCmreDCmreDC-CBCCGTCGATTTAGCGAATATGmreDCtetM-FCAACCCAAATCTGCGACATTTGAGtetRtetM-RCTAAGTTATTTTATTGACAATTGTTATAAACAATCGGATTTAGTACAGCATAspaspa-AA-BamHICCCGGGGATCCAGATTTATGTTATAAACAATCGGATTTAGTACAGCATAspaspa-ABTACTTTCTAGAGAATAAGGAACTTCATTAATACCCCCTGTATGTA	mreDC-AA-BamHI	TTAA <mark>GGATCC</mark> ATCATGATTAAGGCTGAAGACT	mreDC
mreDC-BATATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGTTAmreDCmreDC-BB-SallGATTGTCGACACCACGACCTACATTTCmreDCmreDC-CACAATGCTACAAACCTCCTAATACGCmreDCmreDC-CBCCGTCGATTTTAGCGAATAATGmreDCtetM-FCAACCCAAATCTCGCAATTTGAGtetRtetM-RCTAAGTTATTTATTGAACATATATCTTACTTtetRspa-AA-BamHICCCGGGGATCCAGATTTAGGAACATCATTAATAGTCAACAAACGGATAGTAAGGCATAspaspa-ABTACTTTCTAGAAGAATAGGAACTTCAATAATCGGATTTGTAAAGCAATCACTCspaspa-BATATTCTCTAGAAGAATAGGAACTTCAAACAAACAAACAATACACAACGATAGGATATCATTTATCCspaspa-BB-SallTGCAGGCCGCACTTAGTATGGAGTGCACCATTCTCAAAAAAAA	mreDC-AB	TTACCTATCACCTCAAATGGTTCGCCACCCAGAACACCTCTATTATG	mreDC
mreDC-BB-SallGATTGTCGACACCACGACCACGACCTACATTTCmreDCmreDC-CACAATGCTACAAACCTCCTAATACGCmreDCmreDC-CBCCGTCGATTTTAGCGAATATGmreDCtetM-FCAACCCAAATCTCGCAATTTGAGtetRtetM-RCTAAGTTATTTTATGAACATATATCTTACTTtetRspa-AA-BamHICCCGGGGATCCAGATTTATGTTATAAAACAATCGGATTTAGTACAGCATAspaspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCTGTATGTA	mreDC-BA	TATGTTCAATAAAATAACTTAGAAGATGCAATAGTTGAGTAGTTA	mreDC
mreDC-CACAATGCTACAAACCTCCTAATACGCmreDCmreDC-CBCCGTCGATTTTAGCGAATAATGmreDCtetM-FCAACCCAAATCTCGCAATTTGAGtetRtetM-RCTAAGTTATTTTATTGAACATATATCTTACTTtetRspa-AA-BamHICCCGGGGATCCAGATTTAGGAACATCGGATTAGTACAGCATAspaspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCTGTATGTA	mreDC-BB-Sall	GATT <mark>GTCGAC</mark> ACCACGACCTACATTTTC	mreDC
mreDC-CBCCGTCGATTTTAGCGAATAATGmreDCtetM-FCAACCCAAATCTCGCAATTTGAGtetRtetM-RCTAAGTTATTTTATTGAACATATATCTTACTTtetRspa-AA-BamHICCCGGGGATCCAGATTAGTATAGTATAAACAATCGGATTTAGTACAGCATAspaspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCGTGATGTATTTGTAAAGTCATCspaspa-BATATTCTCTAGAAAGTATAGGAACTTCAAACAAACAATACACAACGATAGATA	mreDC-CA	CAATGCTACAAACCTCCTAATACGC	mreDC
InterportInterporttetM-FCAACCCAAATCTCGCAATTTGAGtetRtetM-RCTAAGTTATTTTATTGAACATATATCTTACTTtetRspa-AA-BamHICCCGGGGATCCAGATTTATGTTATAAACAATCGGATTTAGTACAGCATAspaspa-ABTACTTTCTAGAGAATAGGAACTTCAATAATACCCCCTGTATGTA	mreDC-CB	CCGTCGATTTTAGCGAATAATG	mreDC
tetM-RCTAAGTTATTTTATTGAACATATATCTTACTTtetRspa-AA-BamHICCCGGGGATCCAGATTTATGTTATAGTATATCTTACTTspaspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCTGTATGTA	tetM-F	CAACCCAAATCTCGCAATTTGAG	tetR
Spa-AA-BamHICCCGGGGATCCAGATTATGTTATGTTATAAACAATCGGATTTAGTACAGCATAspaspa-ABTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCTGTATGTA	tetM-R	CTAAGTTATTTATTGAACATATATCTTACTT	tetR
Spartin SummTACTTTCTAGAGAATAGGAACTTCATTAATACCCCCCTGTATGTA	spa-AA-BamHI	CCCGG <mark>GGATCC</mark> AGATTTATGTTATAAACAATCGGATTTAGTACAGCATA	sna
SparingTATTCTCTAGAAAGTATAGGAACTTCAAACAAACAATACACAACGATAGATA	spa-AB	TACTTTCTAGAGAATAGGAACTTCATTAATACCCCCTGTATGTA	spa
Spa-BB-SallTGCAGGTCGACTTAGTATGGAGTGCACCATTCTTCAAAAAATTATTCspaspa-BB-SallTGCAGGCCGAAGTCGATATGGAGTGCACCATTCTTCAAAAAATTATTCspaspa-CAGTCAAGCCTGAAGTCGATATGACTATAAspaspa-CBATCACTAGCAACAATGGTGGTGTTAGCspakan-FRT-FTATTCTCTAGAAAGTATAGGAACTTCGCGAACCATTTGAGGTGATAGGTAAGATTATkanRkan-FRT-RTACTTTCTAGAGAATAGGAACTTCTCCCTAGGTACTAAAACAATTCATCCAGTAAkanRKan ForGCGAACCATTTGAGGTGATAGGTAAGATTATkanRKan RevTCCTAGGTACTAAAACAATTCATCCAGTAAkanRezrA-AA-BamHITTAAGGATCCAACCTAATGAATAAACAACAGCGTCTAAAezrAezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCATATGCTTCTCCTCCTAATezrAezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACATATTGAAGAAGAAGAAGTATezrA	spa-BA	TATTCTCTAGAAAGTATAGGAACTTCAAACAAACAATACAAACGATAGATA	spa
spacespacespacespa-CAGTCAAGCCTGAAGTCGATATGACTATGACTATAAspaspa-CBATCACTAGCAACAATGGTGGTGTTAGCspakan-FRT-FTATTCTCTAGAAAGTATAGGAACTTCGCGAACCATTTGAGGTGATAGGTAAGATTATkanRkan-FRT-RTACTTTCTAGAGAATAGGAACTTCTCCCTAGGTACTAAAACAATTCATCCAGTAAkanRKan ForGCGAACCATTTGAGGTGATAGGTAAGGTAAGATTATkanRKan RevTCCTAGGTACTAAAACAATTCATCCAGTAAkanRezrA-AA-BamHITTAAGGATCCAACCTAATGAATAAACAACAGCGTCTAAAezrAezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCATATGCTTCTCCTCCTAATezrAezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACATATTGAAGAAGAAGTTATezrA	spa-BB-Sall	TGCAGGTCGACTTAGTATGGAGTGCACCATTCTTCAAAAAATTATTC	spa
spar CBATCACTAGCAACAATGGTGGTGTTAGCsparspar CBATCACTAGCAACAATGGTGGTGTTAGCsparkan-FRT-FTATTCTCTAGAAAGTATAGGAACTTCGCGAACCATTTGAGGTGATAGGTAAGATTATkanRkan-FRT-RTACTTTCTAGAGAATAGGAACTTCTCCCTAGGTACTAAAACAATTCATCCAGTAAkanRKan ForGCGAACCATTTGAGGTGATAGGTAAGATTATkanRKan RevTCCTAGGTACTAAAACAATTCATCCAGTAAkanRezrA-AA-BamHITTAAGGATCCAACCTAATGAATAAACAACGGCTCTAAAezrAezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCATATGCTTCTCCTCCTAATezrAezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACAATTGAAGAAGAAGAAGTTATezrA	spa-CA	GTCAAGCCTGAAGTCGATATGACTATAA	spa
spacespacespacespacekan-FRT-FTATTCTCTAGAAAGTATAGGAACTTCGCGAACCATTTGAGGTGATAGGTAAGATTATkan-FRT-RTACTTTCTAGAGAATAGGAACTTCTCCTAGGTACTAAAACAATTCATCCAGTAAKan ForGCGAACCATTTGAGGTGATAGGTAAGATTATKan RevTCCTAGGTACTAAAACAATTCATCCAGTAAezrA-AA-BamHITTAAGGATCCAACCTAATGAATAAACAACAGCGTCTAAAezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCATATGCTTCTCCTCCTAATezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACAATTGAAGAAGAAGTTAT	spa-CB	ATCACTAGCAACAATGGTGGTGTTAGC	spa
Kan FRT-RTACTTTCTAGAGAATAGGAACTTCTCCCTAGGTACTAAAACAATTCATCCAGTAAkanRKan ForGCGAACCATTTGAGGTGATAGGTAAGATTATkanRKan RevTCCTAGGTACTAAAACAATTCATCCAGTAAkanRezrA-AA-BamHITTAAGGATCCAACCTAATGAATAAACAACAGCGTCTAAAezrAezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCATATGCTTCTCCTCCTAATezrAezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACAATTGAAGAAGAAGTTATezrA	kan-FRT-F	TATTCTCTAGAAAGTATAGGAACTTCGCGAACCATTTGAGGTGATAGGTAAGATTAT	kanR
Kan ForGCGAACCATTTGAGGTGATAGGTAAGATTATkanRKan RevTCCTAGGTACTAAAACAATTCATCCAGTAAkanRezrA-AA-BamHITTAAGGATCCAACCTAATGAATAAACAACAGCGTCTAAAezrAezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCATATGCTTCTCCTCCTAATezrAezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACAATTGAAGAAGAAGTTATezrA	kan-FRT-R	TACTTTCTAGAGAATAGGAACTTCTCCTAGGTACTAAAACAATTCATCCAGTAA	kanR
Kan RevTCCTAGGTACTAAAACAATTCATCCAGTAAkanRezrA-AA-BamHITTAAGGATCCAACCTAATGAATAAACAACAGCGTCTAAAezrAezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCATATGCTTCTCCTCCTAATezrAezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACAATATGAAGAAGAAGTTATezrA	Kan For	GCGAACCATTTGAGGTGATAGGTAAGATTAT	kanR
Run RevFormation formation form	Kan Rev	тсстаботастааасааттсатссабтаа	kanR
ezrA-ABATCTTACCTATCACCTCAAATGGTTCGCTATATAACACCCATATGCTTCTCCTCCTAATezrAezrA-BAGGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACATATTGAAGAAGAAGTTATezrA	ezra-Aa-BamHI	ΤΤΑ Α <mark>GG ΑΤΟΓ</mark> Α ΑΓΟΤΑ ΑΤGΑ ΑΤΑ ΑΑΓΑ ΑΓΑ ΑΓΟΤΟΤΑ ΑΑ	ozra
ezrA-BA GGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACATATTGAAGAAGAAGTTAT ezrA	ezrA-AR	ATCTTACCTATCACCTCAAATGGTTCGCTATATAACACACATATGCTTCTCCTCCTAAT	ezrA
	ezrA-BA	GGATGAATTGTTTTAGTACCTAGGACAGGTGTTACTAAACATATTGAAGAAGAAGTTAT	ezrA
ezrA-BR-Sall ATTGTCGACAAAGTTTTCATTAGCTATCTTCATCGC ozrA	ezrA-RR-Sall	ATTGTCGACAAAGTTTTCATTAGCTATCTTCATCGC	ezra
ezrA-CA TGGTTAACTAATTGACGTGCTTGAC ozrA	ezrA-CA	TGGTTAACTAATTGACGTGCTTGAC	ezra
ezrA-CB TGACGGACGTCATTATTTAACTCAGT ezrA	ezrA-CB	TGACGGACGTCATTATTTAACTCAGT	ezrA

Continued Table S2. Primers		
Primers	Sequence $(5 \rightarrow 3)$, restriction site in red	Gene
01649-AA-BamHI	CCCGG <mark>GGATCC</mark> AGCGCATGGTTGTAAAATTTTAACTTTGCA	01649
01649-AB	CAACTCAAATTGCGAGATTTGGGTTGTTAGTCCTCCACTATGCTGCTTGATA	01649
01649-BA	GATAGATAAAGTAAGATATATGTTCAATAAAATAACTTAGATGAGTCGAAAAATAAAT	01649
	ACTTTTATGATGTACAAC	
01649-BB-Sall	TGCAG <mark>GTCGAC</mark> CCTAGAACGATATATTTCGGATTACTTGTA	01649
01649-CA	CAGGTGGAGACTTAGGTAGAGATG	01649
01649-CN	CAATTTCAGTTTCAAATTGAGCAGGTGC	01649
ugtP-gfp-AB	AGCTCCACCAGCGCTACCACCACCTTTAACGAAGAATCTTGCATATAAAGG	ugtP
ugtP-gfp-BA	TAATCTTACCTATCACCTCAAATGGTTCGCTTACGCTAATCATAAAAATTTCATTTTAA	ugtP
ugtP-gfp-BB-HindIII	GGC <mark>AAGCTT</mark> CCTATCATTGAGCCGAATCCTTG	ugtP
ugtP-gfp-CA	ATGGTTACTCAAAATAAAAAGATATTGATTA	ugtP
ugtP-gfp-CB	TTACTTAGCTTTTTCTCTATTTACTATAAAGT	ugtP
ugtP-F-Sall	AAGTT <mark>GTCGAC</mark> CAAACTAACGGAGGGTGGCT	ugtP
ugtP-R-BamHI	GTTTTAA <mark>GGATCC</mark> TTATTTAACGAAGAATCTTGCATATA	ugtP
mNeon-GFP-F	ATGGTGAGTAAAGGTGAGGAGGATAACA	GFP
mNeon -GFP-R	TTATTTATACAACTCATCCCATA	GFP
mNeon -GFP-Km-R	TTACTGGATGAATTGTTTTAGTACCTAGGATTATTTATACAACTCATCCATTCCCATA	GFP
01050-AA-Sall	ATT <mark>GTCGAC</mark> GAATATACAGTTCAA AAATTGAAAAGAGA	01050
01050-AB	TACTGGATGAATTGTTTTAGTACCTAGGACGTTACAACTCCTTATAGTACTTATCCC	01050
01050-tetR-rbs-BA	GTATGATGGTACCATATCGATGAAGGTGAATAAATGACTGGAGAACAATTTACTCAAATT	01050
01050-BB-HindIII	TTAT <mark>AAGCTT</mark> TACGTTTTTCTTTTTTTTAGC	01050
01050-CA	CGGTACACATTATGATGTTGCTTTTC	01050
01050-CB	GTTGTAAATTTGACGTCGCTCTTC	01050
P _{aTc} -Km-F	ATAATCTTACCTATCACCTCAAATGGTTCGCCCGTGAAGTTACCATCACGGAAA	aTc promoter
P _{aTc} -rbs-R	TTATTCACCTTCATCGATATGGTACCATCATACTCTATCAATGATAGAGAGCT	aTc promoter
Apr-F	TTTGCAAGCAGCAGATTACGC	Apr ^R
Apr-R	CGTCATCTCGTTCTCCGCTCAT	Apr ^R
KFC-F	AAGTTGGGTAACGCCAGGGTTTTC	
KFC-R	ATTGTGAGCGGATAACAATTTCACACA	
lpxC-prom-F-SalI	TTTAGCGTACAATCTATTGAAAGGCA	lpxC
lpxC-R-HindIII	TTATGTCACACTCACGTATGGAATTG	lpxC
lpxC-prom-F-KasI	TTTAGCGTACAATCTATTGAAAGGCA	lpxC
lpxC-R-EcoRI	TTATGTCACACTCACGTATGGAATTG	lpxC
adeFGH-AA-Sall	GGC <mark>GTCGAC</mark> GAAGATAAACTGCTGAAATCGGC	adeFGH
adeFGH-AB	GCGTAATCTGCTGCTTGCAAACGGGAAAATGACATGAGGTGCT	adeFGH
adeFGH-BA	ATGAGCGGAGAACGAGATGACGAGGGGGGGGGGGGGGGG	adeFGH
adeFGH-BB-KpnI	TAG <mark>GGTACC</mark> GCTCACAAGCTAACTCTGCTGTTTAT	adeFGH
adeFGH-CA	AGTTCAGCGACCCAATCTACAAAC	adeFGH
adeFGH-CB	AGAACTTTTTGGTGCAGATTACGC	adeFGH

Table S3. Synthetic lethal with <i>∆tarO</i>			
SAOUHSC	Gene name	Source	
00965		Ref. 30, 31	
00618		Ref. 30	
00718		Ref. 30	
00665	graR	Ref. 30, 31	
02611	lyrA	Ref. 30, 31	
00668	vraG	Ref. 30, 31	
00948		Ref. 30	
00953*	ugtP	Ref. 30	
00667	vraF	Ref. 30, 31	
01187	stk1	Ref. 30	
00869	dltA	Ref. 30, 31	
00870	dltB	Ref. 30, 31	
00871	dltC	Ref. 30, 31	
00872	dltD	Ref. 30, 31	
00952*	ItaA	This study	

* manipulated in this study