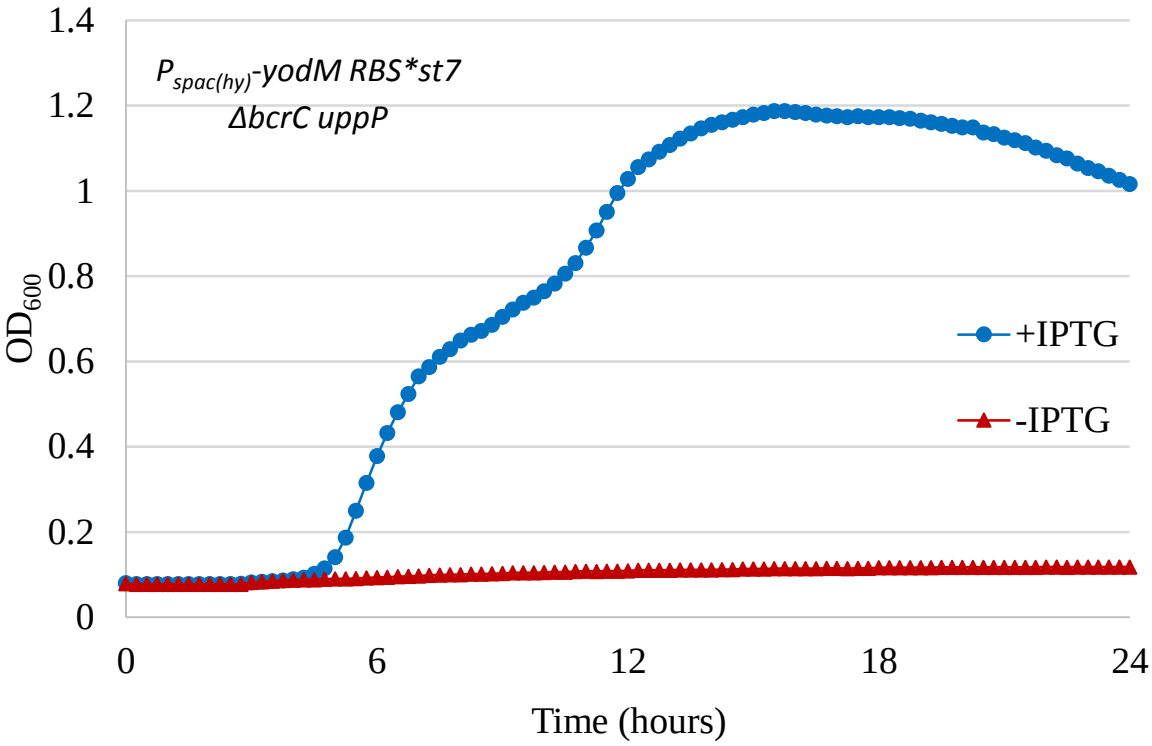

Fig. S1. Overexpression of YodM rescues growth defect of a *uppP bcrC* double mutant. Growth of *yodM* overexpression strain in absence and presence of 1 mM IPTG. An ectopic copy of *yodM* was overexpressed from the *Pspac(hy)* promoter with a strong RBS and ATG as start codon.

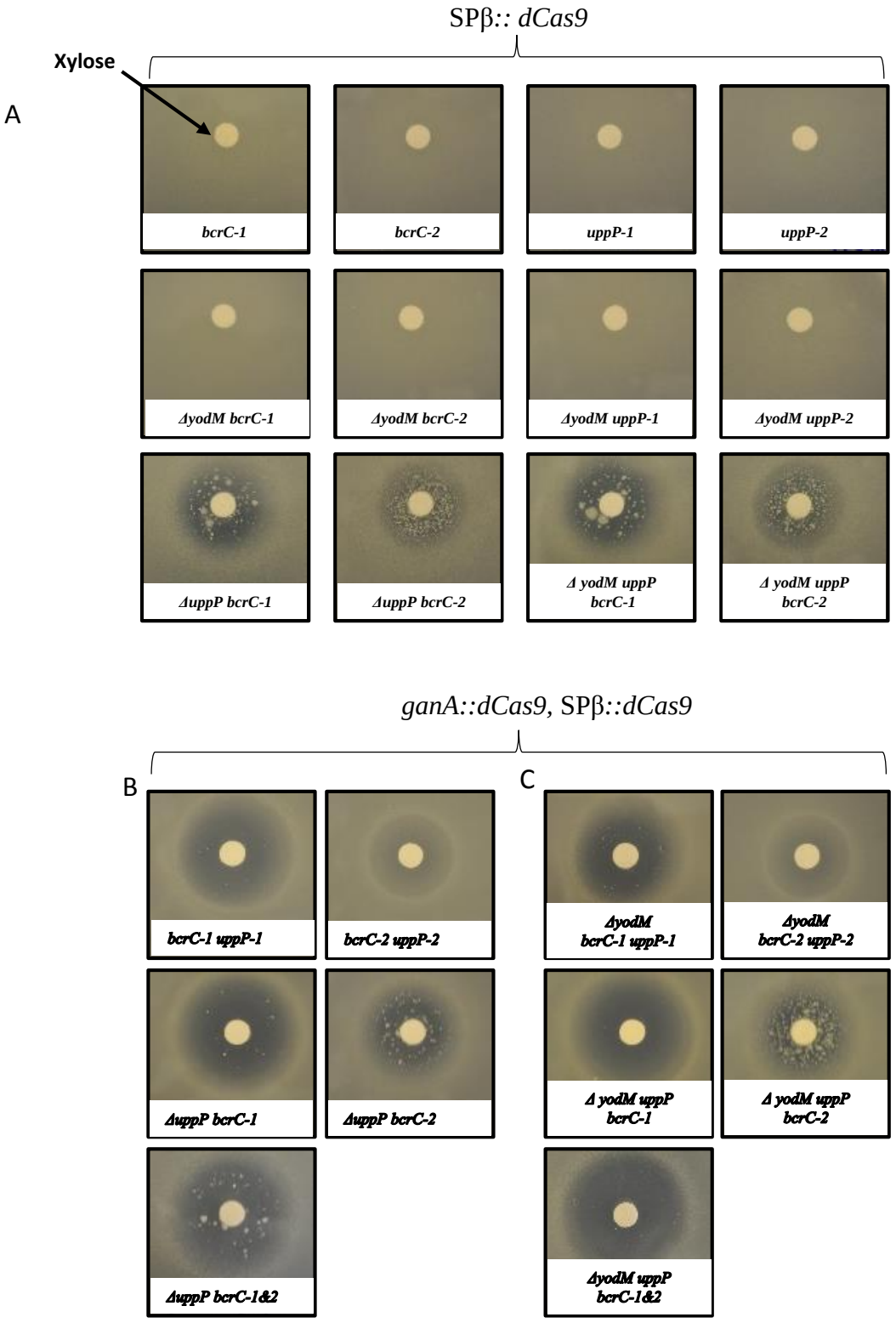

Fig. S2 A dCas9 merodiploid reduces the frequency of spontaneous suppressor mutations. Photos of disk diffusion assay of WT and mutant strains. Cells were grown on LB plates with xylose added onto the filter paper disk.

A. Strains having single *dCas9* in SPβ showed similar phenotype as having single *dCas9* in *ganA* site, but had more of suppressors inside the clear zone.
B. Mutants containing two *dCas9* is more sensitive to induction of *dCas9* by addition of xylose. With chromosomal copy of *yodM* intact, there were still many suppressors inside the clear zone.
C. Mutants having two copies of *dCas9* show similar phenotype in Δ*yodM* background. The number of suppressors is much smaller in *ΔuppP bcrC*-1&2 background lacking *yodM*.

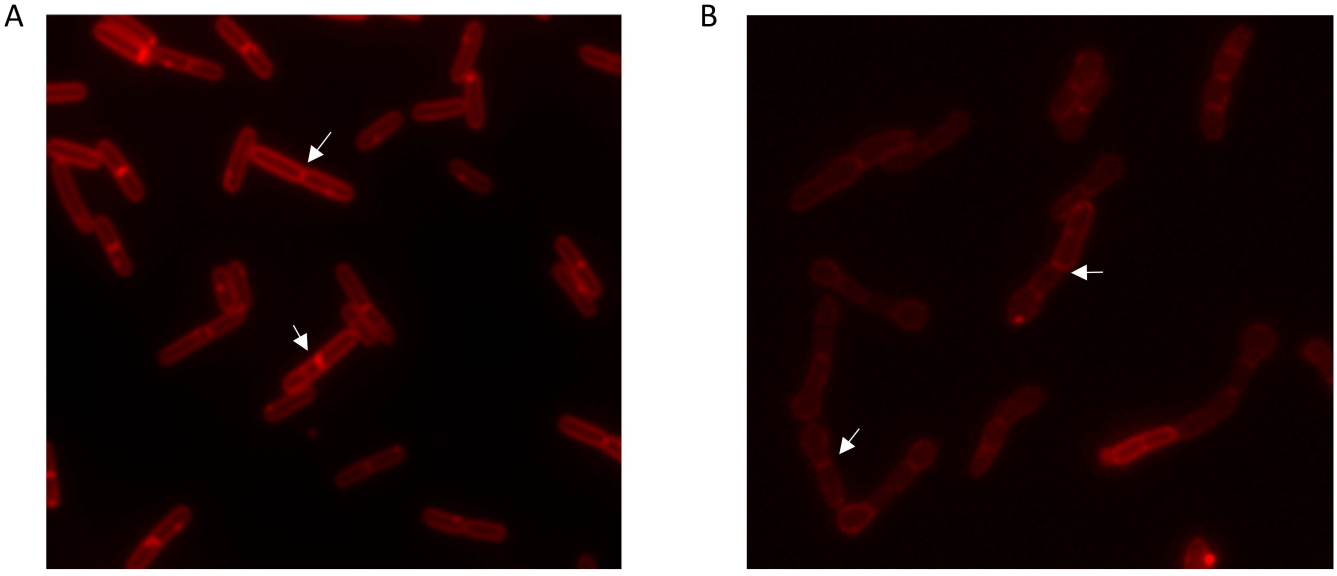

Fig. S3. Membrane stain of WT (A) and optimized depletion strain (B) after growing in presence of xylose. Cells were growing in CH medium containing 2% xylose and membrane was strained by FM5-95. Images were taken 4 hours after addition of xylose. Depletion strain (B) still formed septum (arrows) as WT strain (A) but exhibited bulging phenotype after depletion of UPP-Pases.

Table S1. Homologs of *E.coli* UPP-Pases in *B.subtilis*

***E.coli* UPP-Pase**	***B.subtilis* Homologue**	**Query coverage**	**E Value**	**Identity**
BacA	UppP	93%	5.00E-79	48%
YbjG	BcrC	78%	3.00E-18	30%
	YodM	33%	0.003	36%
PgpB	YodM	23%	0.01	34%
YeiU (LpxT)	BcrC	63%	0.005	23%

Table S2. IPTG-regulated expression of candidate UPP-Pases with altered RBS sequences in a *bcrC uppP* double mutant

Ectopic copy of gene[a]	**RBS***	**Growth**[b]	
$P_{spac(hy)}$-*bcrC*		*+IPTG*	*-IPTG*
Native RBS	aaat**gtaaaagg** *tgattat* ttg	+	+
Native 6[c]	aaat**gtaaaagg** *gattat* ttg	(-)	
Native 5	aaat**gtaaaagg** *attat* ttg	(-)	
Native 4	aaat**gtaaaagg** *ttat* ttg	(-)	
$P_{spac(hy)}$-*uppP*			
Native RBS	aat**ggggagaa** *tcaaaatc* atg	(-)	
Strong[c] 7	**taaggagg** *caaaatc* atg	+	+
Strong 6	**taaggagg** *aaaatc* atg	+	+
Strong 5	**taaggagg** *aaatc* atg	+	+
Strong 4	**taaggagg** *aatc* atg	+	+
$P_{spac(hy)}$-*yodM*			
Native RBS and Start Codon	t**tgaggtgg** *ttaaaa* ttg	(-)	
Strong 7	**taaggagg** *tgattat* atg	+	-

*Predicted Ribosome Binding Site (RBS) is in **bold**, spacer region between RBS and start codon is in *italic*, and start codon is underlined. For purposes of clarity, one or more space was used between RBS, spacer region and start codon.

[a] Transformation deleting *bcrC* and *uppP* in two consecutive steps was performed in strains with ectopic copy of indicated gene. In a successful transformation, resulting strain should miss both chromosomal copy of *bcrC* and *uppP*.

[b] "+" indicates growth and "-" indicates no growth. (-) indicates that the strain could not be constructed due to failure to get transformants even under IPTG-inducing conditions.

[c] Number after "strong" or "native" is the number of base pairs for the spacer region.

Table S3. Percentage of suppressor outgrowth in selected strain backgrounds

Strain Number	Genotype	Number of cultures with outgrowth [a]	Percentage of cultures with outgrowth [b]
HB17177	*ganA*::*dCas9*, *bcrC*-1, *uppP*-1	30	100%
HB17190	*ganA*::*dCas9*, *yodM* null markerless, *uppP* null markerless, *bcrC*-1, *bcrC*-2	30	100%
HB17221	*ganA*::*dCas9*, *SPβ*::*dCas9*, *bcrC*-1, *uppP*-1	22	73.3%
HB17235	*ganA*::*dCas9*, *SPβ*::*dCas9*, *yodM*, *uppP* null markerless, *bcrC*-1, *bcrC*-2	2	6.7%

a. Suppressor outgrowth was monitored using an automated BioScreen growth analyzer. Approximately $4x10^4$ logarithmic phase cells were inoculated into 200 µl of LB medium supplemented with final concentration of 2% (w/v) xylose. 30 replicate cultures (3 biological replicates containing 10 technical replicates each) were used for each strain. Outgrowth is defined as OD_{600}>0.3 after 12 hours of incubation.

b. Percentage of outgrowth cultures was calculated by dividing number of cultures with outgrowth by the total number (30) of cultures.

Table S4. Strains, plasmids and primers used in this study

Strain Number	Genotype
168	Wild Type
ZB307	*trp*$^{+}$ *phe*$^{+}$ SPβc2Δ2::Tn917::pSK10Δ6
HB17042	*bcrC*::MLS
HB17043	*uppp*::spec
HB17044	*yodM*::tet
HB17046	P$_{spac(hy)}$-*uppP*
HB17045	P$_{spac(hy)}$-*bcrC*
HB17047	P$_{spac(hy)}$-*yodM*
HB17071	P$_{spac(hy)}$-*uppP, bcrC*::*MLS*
HB17072	P$_{spac(hy)}$-*uppP, uppP*::*spec*
HB17073	P$_{spac(hy)}$-*yodM, bcrC*::*MLS*
HB17074	P$_{spac(hy)}$-*yodM, uppP*::*spec*
HB17075	P$_{spac(hy)}$-*bcrC*, bcrC*::*MLS, uppP*::*spec*
HB17076	P$_{spac(hy)}$-*bcrC** RBS *wk6*
HB17077	P$_{spac(hy)}$-*bcrC** RBS *wk5*
HB17078	P$_{spac(hy)}$-*bcrC** RBS *wk4*
HB17079	P$_{spac(hy)}$-*uppP** RBS *st7*
HB17080	P$_{spac(hy)}$-*uppP** RBS *st6*
HB17081	P$_{spac(hy)}$-*uppP** RBS *st5*
HB17082	P$_{spac(hy)}$-*uppP** RBS *st4*
HB17083	P$_{spac(hy)}$-*bcrC** RBS *wk6, bcrC*::*MLS*
HB17084	P$_{spac(hy)}$-*bcrC** RBS *wk5, bcrC*::*MLS*
HB17085	P$_{spac(hy)}$-*bcrC** RBS *wk4, bcrC*::*MLS*
HB17086	P$_{spac(hy)}$-*uppP** RBS *st7, bcrC*::*MLS*
HB17087	P$_{spac(hy)}$-*uppP** RBS *st6, bcrC*::*MLS*
HB17088	P$_{spac(hy)}$-*uppP** RBS *st5, bcrC*::*MLS*
HB17089	P$_{spac(hy)}$-*uppP** RBS *st4, bcrC*::*MLS*
HB17090	P$_{spac(hy)}$-*bcrC** RBS *wk6, uppP*::*spec*
HB17091	P$_{spac(hy)}$-*bcrC** RBS *wk5, uppP*::*spec*
HB17092	P$_{spac(hy)}$-*bcrC** RBS *wk4, uppP*::*spec*
HB17093	P$_{spac(hy)}$-*uppP** RBS *st7, uppP*::*spec*
HB17094	P$_{spac(hy)}$-*uppP** RBS *st6, uppP*::*spec*
HB17095	P$_{spac(hy)}$-*uppP** RBS *st5, uppP*::*spec*
HB17096	P$_{spac(hy)}$-*uppP** RBS *st4, uppP*::*spec*
HB17132	*bcrC* null makerless
HB17160	*uppP* null markerless
HB17162	*yodM* null markerless
HB17164	*bcrC, yodM* null markerless
HB17166	*uppP, yodM* null markerless

HB17167	*ganA*::*dCas9*
HB17168	*ganA*::*dCas9, bcrC* null markerless
HB17169	*ganA*::*dCas9, uppP* null markerless
HB17170	*ganA*::*dCas9, yodM* null markerless
HB17171	*ganA*::*dCas9, bcrC, yodM* null markerless
HB17172	*ganA*::*dCas9, uppP, yodM* null markerless
HB17173	*ganA*::*dCas9, bcrC*-1
HB17174	*ganA*::*dCas9, bcrC*-2
HB17175	*ganA*::*dCas9, uppP*-1
HB17176	*ganA*::*dCas9, uppP*-2
HB17177	*ganA*::*dCas9, bcrC*-1, *uppP*-1
HB17178	*ganA*::*dCas9, bcrC*-2, *uppP*-2
HB17179	*ganA*::*dCas9, uppP* null markerless, *bcrC*-1
HB17180	*ganA*::*dCas9, uppP* null markerless, *bcrC*-2
HB17181	*ganA*::*dCas9, uppP* null markerless, *bcrC*-1, *bcrC*-2
HB17182	*ganA*::*dCas9, yodM* null markerless, *bcrC*-1
HB17183	*ganA*::*dCas9, yodM* null markerless, *bcrC*-2
HB17184	*ganA*::*dCas9, yodM* null markerless, *uppP*-1
HB17185	*ganA*::*dCas9, yodM* null markerless, *uppP*-2
HB17186	*ganA*::*dCas9, yodM* null markerless, *bcrC*-1, *uppP*-1
HB17187	*ganA*::*dCas9, yodM* null markerless, *bcrC*-2, *uppP*-2
HB17188	*ganA*::*dCas9, yodM* null markerless, *uppP* null markerless, *bcrC*-1
HB17189	*ganA*::*dCas9, yodM* null markerless, *uppP* null markerless, *bcrC*-2
HB17190	*ganA*::*dCas9, yodM* null markerless, *uppP* null markerless, *bcrC*-1, *bcrC*-2
HB17191	SPβ::*dCas9*
HB17192	SPβ::*dCas9, bcrC* null markerless
HB17193	SPβ::*dCas9, uppP* null markerless
HB17194	SPβ::*dCas9, yodM* null markerless
HB17195	SPβ::*dCas9, bcrC, yodM* null markerless
HB17196	SPβ::*dCas9, uppP, yodM* null markerless
HB17197	SPβ::*dCas9, bcrC*-1
HB17198	SPβ::*dCas9, bcrC*-2
HB17199	SPβ::*dCas9, uppP*-1
HB17200	SPβ::*dCas9, uppP*-2
HB17201	SPβ::*dCas9, bcrC*-1, *uppP*-1
HB17202	SPβ::*dCas9, bcrC*-2, *uppP*-2
HB17203	SPβ::*dCas9, uppP* null markerless, *bcrC*-1
HB17204	SPβ::*dCas9, uppP* null markerless, *bcrC*-2
HB17205	SPβ::*dCas9, uppP* null markerless, *bcrC*-1, *bcrC*-2
HB17206	SPβ::*dCas9, yodM* null markerless, *bcrC*-1
HB17207	SPβ::*dCas9, yodM* null markerless, *bcrC*-2
HB17208	SPβ::*dCas9, yodM* null markerless, *uppP*-1
HB17209	SPβ::*dCas9, yodM* null markerless, *uppP*-2

HB17210	SPβ::*dCas9*, *yodM* null markerless, *bcrC*-1, *uppP*-1
HB17211	SPβ::*dCas9*, *yodM* null markerless, *bcrC*-2, *uppP*-2
HB17212	SPβ::*dCas9*, *yodM* null markerless, *uppP* null markerless, *bcrC*-1
HB17213	SPβ::*dCas9*, *yodM* null markerless, *uppP* null markerless, *bcrC*-2
HB17214	SPβ::*dCas9*, *yodM* null markerless, *uppP* null markerless, *bcrC*-1, *bcrC*-2
HB17216	*ganA*::*dCas9*, SPβ::*dCas9*, *bcrC*-1
HB17217	*ganA*::*dCas9*, SPβ::*dCas9*, *bcrC*-2
HB17218	*ganA*::*dCas9*, SPβ::*dCas9*, *uppP*-1
HB17219	*ganA*::*dCas9*, SPβ::*dCas9*, *uppP*-2
HB17220	*ganA*::*dCas9*, SPβ::*dCas9*, *uppP* null markerless
HB17221	*ganA*::*dCas9*, SPβ::*dCas9*, *bcrC*-1, *uppP*-1
HB17222	*ganA*::*dCas9*, SPβ::*dCas9*, *bcrC*-2, *uppP*-2
HB17223	*ganA*::*dCas9*, SPβ::*dCas9*, *uppP* null markerless, *bcrC*-1
HB17224	*ganA*::*dCas9*, SPβ::*dCas9*, *uppP* null markerless, *bcrC*-2
HB17225	*ganA*::*dCas9*, SPβ::*dCas9*, *uppP* null markerless, *bcrC*-1, *bcrC*-2
HB17226	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM* null markerless, *bcrC*-1
HB17227	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM* null markerless, *bcrC*-2
HB17228	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM* null markerless, *uppP*-1
HB17229	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM* null markerless, *uppP*-2
HB17230	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM*, *uppP* null markerless
HB17231	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM* null markerless, *bcrC*-1, *uppP*-1
HB17232	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM* null markerless, *bcrC*-2, *uppP*-2
HB17233	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM*, *uppP* null markerless, *bcrC*-1
HB17234	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM*, *uppP* null markerless, *bcrC*-2
HB17235	*ganA*::*dCas9*, SPβ::*dCas9*, *yodM*, *uppP* null markerless, *bcrC*-1, *bcrC*-2
HB17236	$P_{spac(hy)}$-*yodM** RBS st7
HB17237	$P_{spac(hy)}$-*yodM** RBS st7, *bcrC* null markerless
HB17239	$P_{spac(hy)}$-*yodM** RBS st7, *bcrC* null markerless, *uppP*::*spec*

Plasmid	**Description**
pPL82	Integration plasmid into *amyE* site
pJMP1	Integration plasmid containing *dCas9* into *ganA* site
pJMP2	Integration plasmid for sgRNA into *amyE* site
pJMP3	Integration plasmid for sgRNA into *thrC* site
pJPM122	Integration plasmid into modified SPβ phage strain ZB307
pSPβ::*dCas9*	Integration plasmid containing *dCas9* into modified SPβ phage strain ZB307
pJMP2-*bcrC*-2	pJMP2 with sgRNA *bcrC*-2
pJMP3-*bcrC*-1	pJMP3 with sgRNA *bcrC*-1
pJMP3-*uppP*-2	pJMP3 with sgRNA *uppP*-2
pJMP2-*uppP*-1	pJMP2 with sgRNA *uppP*-1

Primer Number	**Name**	**Sequence**
6048	*bcrC*-up-for	ACTTAACGATGCACGGGGAA

6049	*bcrC*-up-rev(mls)	GAGGGTTGCCAGAGTTAAAGGATCCCATGGATTGCTTTAAAAATTTCGT
6050	*bcrC*-down-for(mls)	CGATTATGTCTTTTGCGCAGTCGGCCGTGAGGATCTACGAAGCCA
6051	*bcrC*-down-rev	AGTGAAGACAGCGGAAACCA
6186	*uppP* up F	ACGATCGTGAACAGCACCTT
6189	*uppP* down R	TCCAAGACATTTTTGGCGGC
6221	*bcrC* depletion F XmaI	GATCCCCGGGTGTAAAAGGTGATTATTTGAACTAC
6222	*bcrC* depletion R XbaI	GATCTCTAGACTGTCTTGATTTCAGACGCC
6223	*uppP* depletion F XmaI	GATCCCCGGGTAATGGGGAGAATCAAAATC
6224	*uppP* depletion R XbaI	GATCTCTAGAAGGCTCGGAAAAGAGCCTTA
6233	*uppP* up R sepc	CGTTACGTTATTAGCGAGCCAGTCGGCGGCTACAAACAATTCCC
6234	*uppP* down F spec	CAATAAACCCTTGCCCTCGCTACGACTTGTCCCATTTGCAATCTATCG
6235	*yodM* Up F	GAGTGGCTTGAAACGGAGGA
6236	*yodM* up R tet	GAGAACAACCTGCACCATTGCAAGAAGAAACAAACTAACGGGCTTGT
6237	*yodM* Down F tet	GGGATCAACTTTGGGAGAGAGTTCCGAAAAGATTAAGCGGTTTCGAC
6238	*yodM* Down R	AAGGCGCAAAATCAACGCTT
6239	*yodM* F XmaI	GATCCCCGGGGCGGTCAGCTCCGGTTTTATT
6240	*yodM* R XbaI	GATCTCTAGAGCGTTTTTGCTGTGTTTTCCT
6254	*uppP* depletion F HindIII (st7)	GATCAAGCTTTAAGGAGGCAAAATCATGACTCTATGGGAATTG
6255	*uppP* depletion F HindIII (st6)	GATCAAGCTTTAAGGAGGAAAATCATGACTCTATGGGAATTGTTT
6256	*uppP* depletion F HindIII (st5)	GATCAAGCTTTAAGGAGGAAATCATGACTCTATGGGAATTGTTTG
6257	*uppP* depletion F HindIII (st4)	GATCAAGCTTTAAGGAGGAATCATGACTCTATGGGAATTGTTTG
6258	*bcrC* depletion F HindIII (wk6)	GATCAAGCTTTGTAAAAGGGATTATTTGAACTACGAAATTTTTAAAGC
6259	*bcrC* depletion F HindIII (wk5)	GATCAAGCTTTGTAAAAGGATTATTTGAACTACGAAATTTTTAAAGC
6260	*bcrC* depletion F HindIII (wk4)	GATCAAGCTTTGTAAAAGGTTATTTGAACTACGAAATTTTTAAAGC
6411	CRISPRi *bcrC*-1 F	AATTGTGATGAGATAGTCCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
6412	CRISPRi *bcrC*-2 F	TTCCGTGATGAAGACCATAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
6413	CRISPRi *uppP*-1 F	CACGATAGCTACTGCTAAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
6414	CRISPRi *uppP*-2 F	AGCCCAGTAAATTTAAAATTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
6415	CRISPRi universal R	ACATTTATTGTACAACACGAGCC
6424	CRISPRi check F	TGACAAAAATGGGCTCGTGT
6425	CRISPRi check R	ACTTCTGAGTTCGGCATGGG
6426	*yodM* F HindIII (RBS st7)	GATCAAGCTTTAAGGAGGTGATTATATGTACAAGCCCGTTAGTTTGTTTC
6429	*dCas9* check F	CCGTCGTTGGAACTGCTTTG
6430	*dCas9* check R	AGCATCCGTTTACGACCGTT
6326	BKE MLS check R	TTTTCTCGTTCATAGTAGTTCCTCC