

Supplementary information to

“BglJ-RcsB heterodimers relieve repression of the *Escherichia coli* *bgl* operon by H-NS”

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The supplement includes a brief description of construction of plasmids, as well as supplementary tables and figures. Tables S1 and S2, respectively, describe the plasmids and oligonucleotides used in this study. Figure S1 shows the comparison of *E. coli* RcsA-RcsB binding sites with the BglJ-RcsB binding site, figure S2 shows the LeuO DNase I footprint, and figure S3 schematically shows the construction of LexA-two-hybrid reporter plasmids.

Construction of plasmids

Plasmids were constructed according to standard cloning techniques (1), with brief descriptions given in the following. All cloned fragments generated by PCR were sequenced.

Plasmid pKES148 is a vector for cloning of promoter *lacZ* fusions and subsequent integration of these fusions into the phage Lambda *attB* site, as described (5,7). The plasmid carries a multiple cloning site upstream of the promoter-less *lacZ* gene. In addition, the plasmid carries a cassette with the phage lambda *attP* sequence and gene *aadA* conferring spectinomycin resistance for integration into *attB* and selection of integrants. The plasmid backbone consist of a p15A replication origin and the *neo* gene for selection on kanamycin.

The construction of plasmids pKES163 and pKES164 carrying the *sulA* promoter *lacZ* fusions for monitoring interaction of proteins with the LexA-based two-hybrid system is described in Figure S3.

The RcsB-HA expression vector pKEAP38 carries the *rscB* gene under control of *lacI^f tac* promoter cassette. followed by *rnnB* terminators T1 and T2, the *bla* gene for ampicillin resistance and a pMB1 origin of replication. For construction of this plasmid an *rscB* fragment was amplified by PCR using oligonucleotides S684 (5' end) and S683 (3' end). The reverse primer S683 included a sequence encoding an HA-tag. The *rscB*-HA fragment was digested with BglII and XhoI and cloned into plasmid pKEAP22 (Table S1).

Plasmid pKES182 is a vector for fusion of a FLAG tag to the 3' end of an open reading frame. The plasmid was derived from pKESK22 by modification of the promoter and multiple cloning site. Expression is directed by a *lacI^f tac* promoter cassette and the strong Shine-Dalgarno sequence derived from phage T7 gene 10. (The relevant sequence of the promoter and multiple cloning site is: (TTGACAATTA ATCATCGGCT CGTATAATGT GTGGAATTGT GAGCGGATAA CAATTTCA CG AATTCATGG AGATCTAATA *ATTTTGTTTA ACTTTAAGAA GGAGATATAC ATATGTCTAG* AGCGGTCTCA gattacaagg atgacgacga taagTAACTG CAGGATCC, with the -35 and -10 box of the *tac* promoter underlined, the enhanced Shine-Dalgarno sequence in italics and underlined, and the FLAG tag coding sequence in lower case letters). The plasmid carries a p15A origin of replication and confers kanamycin resistance.

Plasmid pKERV10 for expression of a BglJ-FLAG fusion protein was constructed using plasmid pKES182 as vector and oligonucleotides S320 and S887 for cloning of *bglJ*.

Plasmid pKETS1 is a vector for expression of moderate levels of BglJ. This pKESK22 derived plasmid (p15A origin of replication, *neo*) was constructed by insertion of a *bglJ* fragment generated by PCR followed by digestion with EcoRI and XbaI. In contrast, plasmids pKETS9 and pKETS10 carry shorter variants of the *bglJ* open reading frame including the second and third possible start codon of *bglJ* only. As induction of *bglJ* expression does not result in activation of the *bgl* operon, we conclude that translation of *bglJ* starts at the most 5' AUG codon mapping within *yjjQ*, and that *bglJ* maps at position 4602183 to 4602860 in the *E. coli* K12 MG1655 genome sequence and codes for a protein of 225 amino acids.

Plasmids pKETS6, pKETS7, pKETS8, and pKES235 were constructed for expression of RcsB, and RcsB mutants D56E, D56N and D56A. These plasmids carry the *rscB* wild-type gene or the mutants

under control of the *tac* promoter. The plasmids were constructed by cloning *rcsB* fragments which were generated by PCR using the oligonucleotides described in Table S2 into the EcoRI, and XbaI digested vector pKESK22.

Plasmids pKES220, pKES221, and pKES222 were derived from pKENV61 (10) by overlap PCR using oligonucleotides T343 and T344 for BglJ-RcsB binding-site mutant 1, T345 and T353 for mutant 2, and T347 and T354 for mutant 3.

The various LexA fusions plasmids (pKEMK17, pKEAP27, pKEAP30, pKES192, pKEAP28, pKES150, pKES151, and pKEAP29) were constructed using the oligonucleotides described in Table S2.

Figures and Tables


<i>cps</i> (-112 to -99)	<u>TAAAGAAACTCCTA</u>
<i>rcaA</i> (-133 to -120)	<u>TAAGGATTATCCGA</u>
<i>yjb</i> (-154 to -141)	<u>TGAGGTTAATCCTA</u>
<i>flhDC</i> (+5 to +18)	<u>TAGGAAaaaTCctA</u>
<i>flhDC</i> (inverted)	<u>TaaGAtttTTCCTA</u>
RcsAB box	TaAGaatatTCctA
	
<i>bgl</i> (-113 to -100)	<u>TTTATAAAATTCCTA</u>
BglJ-RcsB site	--BglJ- -RcsB--

Figure S1. Comparison of the BglJ-RcsB binding site mapping in the *bgl* regulatory region with RcsA-RcsB binding-sites and the RcsA-RcsB consensus sequence (RcsAB box). The comparison suggests that RcsB contacts the right half of the RcsA-RcsB binding sites as well as of the putative BglJ-RcsB binding site. In the individual RcsA-RcsB binding sites, the matches to the RcsAB box consensus sequence (14) are underlined. The RcsA-RcsB binding site in *flhDC* in inverted orientation matches the consensus sequence better than in direct orientation. In this sequence mutations which prevent binding of RcsA-RcsB heterodimers but not of RcsB (8) are shown in lower case letters. The RcsAB-box Logo was created using MEME (2) with the sequences of the four RcsA-RcsB binding sites (*cps*, *rcaA*, *yjb*, and *flhDC* inverted). The positions of the RcsAB box relative to the transcription start of the respective promoter is shown in brackets and was extracted from the EcoCyc database (9).

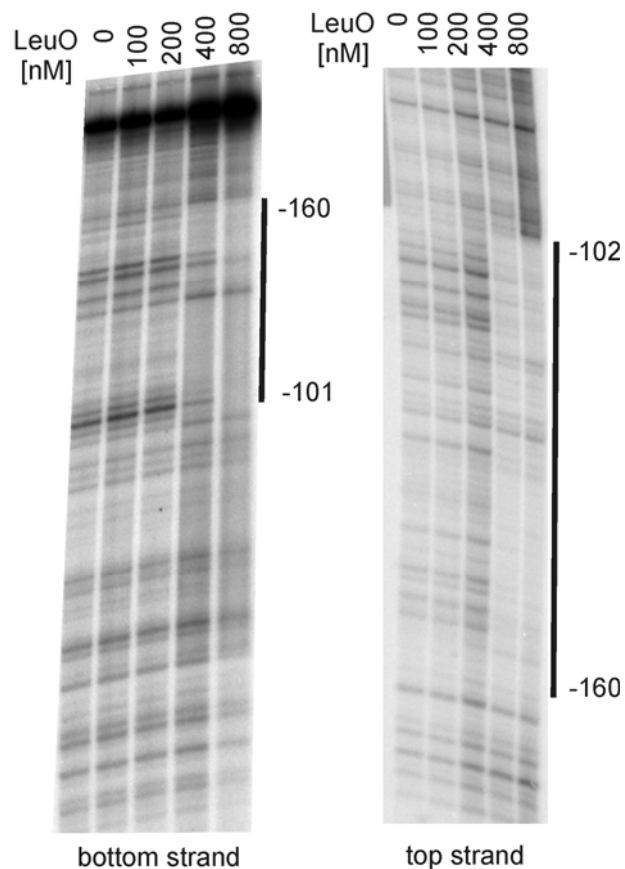


Figure S2. LeuO binds to the *bgl* upstream regulatory element. DNase I footprinting of a *bgl* fragment (position -202 to +29, relative to the transcription start) encompassing the upstream regulatory element and promoter. For footprinting of the top strand the fragment was amplified by PCR using the non-phosphorylated forward primer S79 and the phosphorylated reverse primer T110. For labeling of the bottom strand oligonucleotides S80 (phosphorylated) and T109 (5'-OH) were used. Details of binding and DNase I treatment are given in Material and Methods. The concentration of LeuO used in the reactions is indicated. The samples were separated on a denaturing acrylamide gel next to a sequencing ladder (not shown) as size standard.

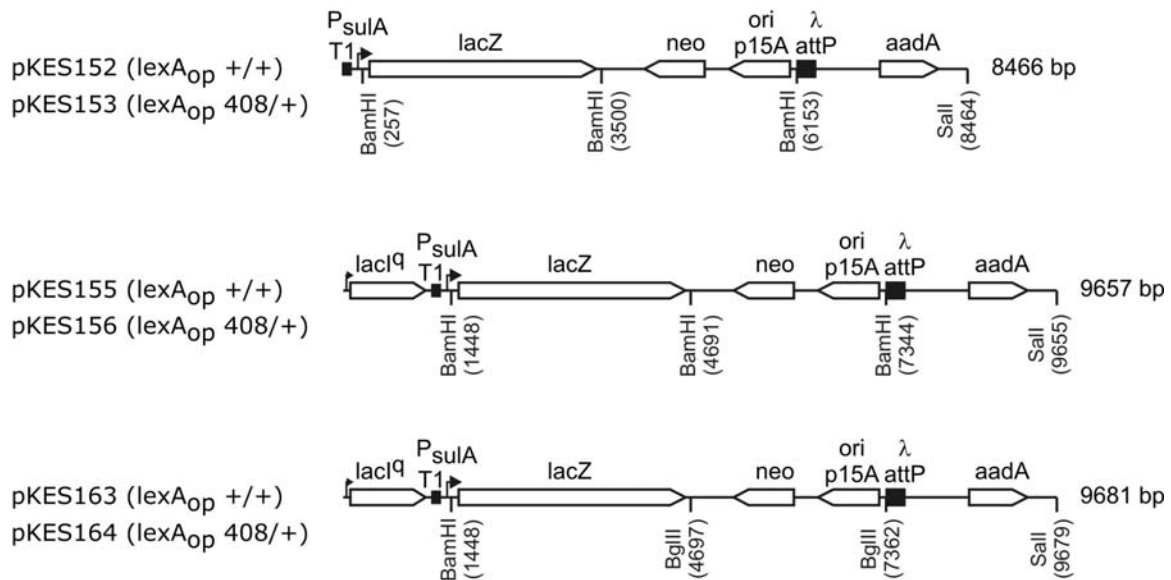
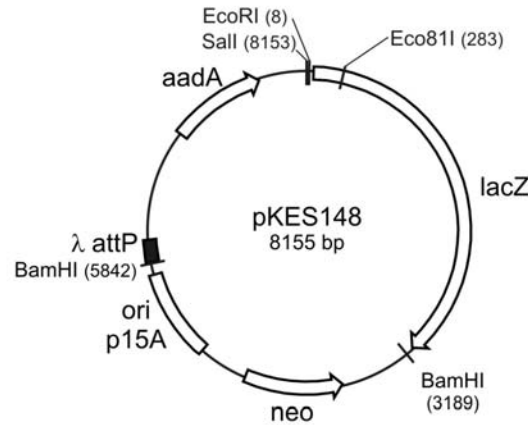
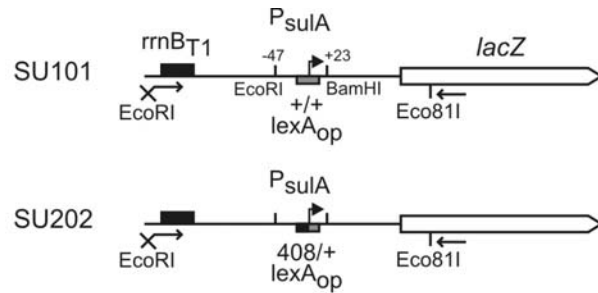


Figure S3. Construction of reporter strains for monitoring interaction of RcsB, BglJ, and YjjQ using the LexA-based one and two hybrid system.

Analysis of the interaction of transcription factors RcsB, BglJ, YjjQ, and RcsA was performed in strains in which the *rcsB* and *yjjQ-bglJ* loci were deleted. However, for unknown reasons we were not able to transduce strains SU101 and SU202 (6) carrying the one- and two-hybrid *sulA* promoter-*lacZ* reporter fusions, and to manipulate the strains using the phage λ *red-gam* based system (4). Therefore, the *sulA* promoter-*lacZ* reporter fusions were cloned and then integrated into the chromosome of strain S3384, which is a *sulA3 lexA71::Tn5 ΔrcsB_{FRT} Δ(yjjP-yjjQ-bglJ)_{FRT}* derivative of strain S541 (CSH50

$\Delta lacZ \Delta bgl thi ara$). In a first step, the *sulA* promoter fragments were amplified from strains SU101 und SU202 using primers S557 and S100, the PCR fragments were digested with Eco81I and EcoRI and cloned into Eco81I and EcoRI digested pKES148, yielding plasmids pKES152 and pKES153. Plasmid pKES148 carries a p15A origin of replication, genes *neo* and *aadA* which confer kanamycin and spectinomycin resistance, respectively, a phage $\lambda attP$ site and a promoterless *lacZ* gene. In a second cloning step a fragment carrying the *lacI^f* allele (generated by PCR with oligonucleotides S275 and S828, followed by Sall-XhoI digestion) was inserted into the Sall site located upstream of the *rrnB-T1 sulA* promoter region, yielding plasmids pKES155 and pKES156. In a third step the vector backbone was replaced by a similar vector backbone in which the two BamHI sites were converted to BglII sites by insertion of 8 bp BglII oligonucleotide linker sequences. This was necessary, since an additional BamHI site maps next to the *sulA* promoter. The resulting plasmids pKES163 and pKES164 were used for integration at the $\lambda attB$ site. Of these plasmids BglII fragments lacking the origin of replication and the *neo* gene were isolated, circularized by ligation, and recombined into the chromosomal *attB* site with helper plasmid pLDR8, as described (5). The resulting strains were S3434 (which is S3384 *attB::(SpecR lacI^f rrnB-T1 PsulA-lexA_{op}wt lacZ*; pKES163) and S3442 (S3384 *attB::(SpecR lacI^f rrnB-T1 PsulA-lexA_{op}408/wt lacZ*; pKES164).

Table S1: Plasmids		
pCP20	cl ₈₅₇ λ-P _R flp in pSC101 rep ^{ts} bla	(3)
pKD3	FRT-cat-FRT oriRy bla	(4)
pKD4	FRT-neo-FRT oriRy bla	(4)
pKD46	araC P _{ara} γ-β-exo in pSC101-ori rep ^{ts}	(4)
pLDR8	cl ₈₅₇ P _R λ-int in ori-pSC101 rep ^{ts} neo	(5)
pFDX733	bgl operon in ori-p15A neo	(12)
pKESD8	P _{bgl} t1 bglG lacZ in ori-p15A neo λ-attP aadA (Spec ^R)	(7)
pKESK22	lacI ^q P _{tac} MCS in ori-p15A neo	(13)
pKEDR13	lacI ^q P _{tac} leuO in ori-p15A neo	(13)
pKETS1	lacI ^q P _{tac} bglJ (NC_000913 position 4602168-4602867) in ori-p15A neo	this work
pKETS9	lacI ^q P _{tac} bglJ (NC_000913 position 4602217-4602867) in ori-p15A neo	this work
pKETS10	lacI ^q P _{tac} bglJ (NC_000913 position 4602313-4602867) in ori-p15A neo	this work
pKETS6	lacI ^q P _{tac} rcsB in ori-p15A neo	this work
pKETS7	lacI ^q P _{tac} rcsB-D56E in ori-p15A neo	this work
pKETS8	lacI ^q P _{tac} rcsB-D56N in ori-p15A neo	this work
pKES235	lacI ^q P _{tac} rcsB-D56A in ori-p15A neo	this work
pKES148	vector for transcriptional lacZ fusions in ori-p15A neo λ-attP aadA (Spec ^R)	this work
pKES152	rrnB-T1 P _{sulA} lexA-op+/+ lacZ in ori-p15A neo λ-attP aadA	this work
pKES153	rrnB-T1 P _{sulA} lexA-op408/+ lacZ in ori-p15A neo λ-attP aadA	this work
pKES154	lacI ^q MCS lacZ in ori-p15A neo attP aadA	this work
pKES155	lacI ^q rrnB-T1 P _{sulA} lexA-op+/+ lacZ in ori-p15A neo λ-attP aadA	this work
pKES156	lacI ^q rrnB-T1 P _{sulA} lexA-op408/+ lacZ in ori-p15A neo λ-attP aadA	this work
pKES163	lacI ^q rrnB-T1 P _{sulA} lexA-op+/+ lacZ in ori-p15A neo λ-attP aadA	this work
pKES164	lacI ^q rrnB-T1 P _{sulA} lexA-op408/+ lacZ in ori-p15A neo λ-attP aadA	this work
pMS604	P _{lacUV5} lexA ₁₋₈₇ -fos in ori-pMB1 tet	(6)
pKEMK17	P _{lacUV5} lexA ₁₋₈₇ -rcsB in ori-pMB1 tet	this work
pKEAP27	P _{lacUV5} lexA ₁₋₈₇ -yjjQ in ori-pMB1 tet	this work
pKEAP30	P _{lacUV5} lexA ₁₋₈₇ -bglJ in ori-pMB1 tet	this work
pKES192	P _{lacUV5} lexA ₁₋₈₇ -rcsA in ori-pMB1 tet	this work
pDP804	P _{lacUV5} lexA408 ₁₋₈₇ -Jun in ori-p15A bla	(6)
pKEAP28	P _{lacUV5} lexA408 ₁₋₈₇ -rcsB in ori-p15A bla	this work
pKES150	P _{lacUV5} lexA408 ₁₋₈₇ -rcsB _{D56E} in ori-p15A bla	this work
pKES151	P _{lacUV5} lexA408 ₁₋₈₇ -rcsB _{D56N} in ori-p15A bla	this work
pKEAP29	P _{lacUV5} lexA408 ₁₋₈₇ -bglJ in ori-p15A bla	this work
pKEAP22	lacI ^q P _{tac} MCS in ori-pMB1 bla	this work
pKEAP38	lacI ^q P _{tac} rcsB-HA in ori-pMB1 bla	this work
pKES182	lacI ^q P _{tac} T7gene10ε-RBS NdeI,XbaI-FLAG ori-p15A neo	this work
pKERV10	lacI ^q P _{tac} T7gene10ε-RBS-bglJ-FLAG ori-p15A neo	this work
pKEAP21	lacI ^q P _{tac} leuO-His ₆ in ori-pMB1 bla	(13)
pKENV61	P _{bgl} t1 _{RAT} bglG lacZ in ori-p15A neo λ-attP aadA	(10)
pKES220	P _{bgl} -mut2 t1 _{RAT} bglG lacZ in ori-p15A neo λ-attP aadA	this work
pKES221	P _{bgl} -mut3 t1 _{RAT} bglG lacZ in ori-p15A neo λ-attP aadA	this work
pKES222	P _{bgl} -mut1 t1 _{RAT} bglG lacZ in ori-p15A neo λ-attP aadA	this work

^a ori-p15A, pro-pMB1, ori-pSC101 rep^{ts}, and oriRy designate the origin of replication of the respective plasmid.

^b MCS (multiple cloning site)

^c T7gene10ε-RBS is the very efficient translation start derived from phage T7 gene 10 (11)

Table S2: Oligonucleotides		
No	sequence	description
S100	CATCGTAACCGTGCATCTGCCA	<i>sulA</i> promoter <i>lacZ</i> fusion (<i>lacZ</i> reverse)
S275	actgctgacACCATCGAATGGTGCAA	<i>lacI^o</i> promoter (Sall)
S320	ggccatgGAACACAGCCGAATTAAGAAGA	<i>bglJ</i> 5' (NdeI)
S323	ggcccatgTTGCCAGGATGCTGCAA	<i>yjjQ</i> 5' (NdeI)
S557	cggaattcTAAACGAAAGGCTCAGTCGAAAG	<i>sulA</i> promoter <i>lacZ</i> fusion (EcoRI)
S665	TCTATTATTACCTAACAAACCACCCCAATATAAGTTTGAGATTACTACAggtga ggctggagctgctcg	Δ hns::kan _{KD4}
S672	AAATCCCGCCGCTGGCGGGATTTTAAGCAAGTGCAATCTACAAAAGATTAc atgaatatcctccttagtcttattcc	Δ hns::kan _{KD4}
S676	GAAAGCACTGCCGGGAAGTAAACCCGGCATCATGCGGATTAc atgaatatcctccttagtcttattcc	Δ (<i>yjjP-yjjQ-bglJ</i>)::cat _{KD3}
S683	agctcgagctagcttaAGCGTAATCTGGAACATCGTATGGGTAGTCTTTATCTGCCG GACTTAAGGTC	<i>rbsB</i> -HA 3' (XhoI)
S684	cgaattcagatctTTGCTGTAGCAAGGTAGCCTATTACAT	<i>rbsB</i> 5' (EcoRI, BglII)
S686	ttctgcagGAACACAGCCGAATTAAGAAGAGAAATG	<i>lexA</i> ₁₋₈₇ - <i>bglJ</i> (PstI)
S687	ttctgcagatctGATTGTACGCTGAAATGAAAGCACTG	<i>lexA</i> 408 ₁₋₈₇ - <i>bglJ</i> (BglII, XhoI)
S688	ttctgcagGAACACAGCCGAATTAAGAAGAGAAATG	<i>lexA</i> wt/408 ₁₋₈₇ - <i>bglJ</i> (XhoI)
S689	ttctgcagAACAAATATGAACGTAATTATTGCCGATG	<i>lexA</i> 408 ₁₋₈₇ - <i>rbsB</i> (XhoI)
S690	ttagatctAATCGGTGCAAATGCCAGATAAG	<i>lexA</i> 408 ₁₋₈₇ - <i>rbsB</i> (BglII)
S691	ttctgcagTTGCCAGGATGCTGCAAAA	<i>lexA</i> ₁₋₈₇ - <i>yjjQ</i> (PstI)
S692	ttctgcagACTCTCAATACCGATACTACTCATGACG	<i>lexA</i> ₁₋₈₇ - <i>yjjQ</i> (XhoI)
S770	GTTGATTACCGAgCTCTCCATGCCTGGCGA	<i>rbsB</i> -D56E
S771	GCATGGAGAGcTCGGTAATCAACACATGCG	<i>rbsB</i> -D56E
S772	TGTTGATTACCAtICTCTCCATGCCTGGCG	<i>l</i> <i>rbsB</i> -D56N
S773	GGCATGGAGAGAtIGGTAATCAACACATGCCCATC	<i>rbsB</i> -D56N
S774	ATTGACAGTTATGTCAAGAGCTTGCTGTAGCAAGGTAGCCTATTACggacagaa atgctcagactcg	Δ <i>rbsB</i> ::specR
S775	TGCCAGATAAGACACTAACGCGTCTTATCTGGCCTACAGGTGATTAtgcttagtg catctaacgcttagt	Δ <i>rbsB</i> ::specR
S783	GAGGATCATATCTGCGCCAACGCTAACAGAAATTCGATC Aggttaggctggagctgctcg	Δ (<i>yjjP-yjjQ-bglJ</i>)::cat _{KD3}
S819	ATTGACAGTTATGTCAAGAGCTTGCTGTAGCAAGGTAGCCTATTACggttaggct ggagctgctcg	Δ <i>rbsB</i> ::cat _{KD3}
S820	TGCCAGATAAGACACTAACGCGTCTTATCTGGCCTACAGGTGATTAc atcctccttagtcttattcc	Δ <i>rbsB</i> ::cat _{KD3}
S828	cggactcgagAGCTAATTACATTAATTGCGTTGCC	<i>lacI</i> reverse (XhoI)
S887	ccactctagaATAGGGATGCAACACATTACTTGTTC	<i>bglJ</i> 3' (XbaI for FLAG tag fusion)
S890	cctatctagaTGAGTGCACATTTCTCTTCTTAATTC	<i>yjjQ</i> 3' (XbaI for FLAG tag fusion)
S927	tgctcagagAATCGGTGCAAATGCCAGATAAG	<i>lexA</i> ₁₋₈₇ - <i>rbsB</i> (XhoI)
S948	tgctggtgaccTCAACGATTATTATGGATTTATGTAGTTACAC	<i>lexA</i> ₁₋₈₇ - <i>rbsA</i> (Eco911)
S949	acgactcgagGGAAACCACAGTCAGAATGTG	<i>lexA</i> ₁₋₈₇ - <i>rbsA</i> (XhoI)
T51	ccatggtgaccAACAAATATGAACGTAATTATTGCCGATG	<i>lexA</i> ₁₋₈₇ - <i>rbsB</i> (Eco911)
T79	GGCGATGAGCTGGATAAACTGCT	<i>bgl</i> -202 to -180
T80	ATGGTTTTTATAACGAACATCCAGGTTT	<i>bgl</i> +29 to +2
T96	GTTACCAGCATTACAGCAGCCGTTAAATATCAATGACTGGCAGCAACACTGC GCGCAGCTGC	T96/T97 are self complementary and were used to restore <i>ilvG</i>
T97	GCAGCTGCGCGCAGTGTGCTGCCAGTCATTGATATTTAACGGCTGCTGTA ATGCTGGTAAC	see T96
T106	CAGGGATCCTCTAGATTAGTCTTTATCTGCCGGACTTAAGGTCAC	<i>rbsB</i> 3' BamHI
T109	5'phosphate GGCGATGAGCTGGATAAACTGCT	<i>bgl</i> -202 to -180
T110	5'phosphate ATGGTTTTTATAACGAACATCCAGGTTT	<i>bgl</i> +29 to +2

Table S2: Oligonucleotides		
No	sequence	description
T207	<u>gaccgaattc</u> TGGAGATGCCGCAGAATGG	bglJ 5' (1 st AUG, EcoRI)
T208	ctggtctagaATGCGGATTAAATAGGGATGCAA	bglJ 3' XbaI
T209	ATTCCAATAAGGGAAAGGGAGTTAAGTGTGACAGTGGAGTTAAGTgtgtaggctg gagctgcttcg	Δ leuO::cat _{KD3}
T210	TGCAGAATAAACCCAGACATTCATGTCTGACCTATTCTGCAATCAGcatatgaatat cctccttagttcctattcc	Δ leuO::cat _{KD3}
T343	GTTATATATAACTTTATAAA <u>Tag</u> CTAAAATTACACAAAGTTAATAACTGCGAGC	BglJ-RcsB site mutant 1
T344	ATTAAC ^T TTTGTGTAATTTT <u>AG</u> GctATTTATAAAAGTTATATATAACAAATCCCAATA ATTAAGTTA	BglJ-RcsB site mutant 1
T345	TGTTATATATAACGTTtcAAATTCCTAAAATTACACAAAGTTAATAACTGC	BglJ-RcsB site mutant 2
T347	GTTATATATAACGTTtcAAAT <u>Tag</u> CTcAAATTACACAAAGTTAATAACTGCGAGCA	BglJ-RcsB site mutant 3
T353	TGTAATTTT <u>AGGA</u> ATTTgaAAcGTTATATATAACAAATCCCAATAATTAAGTTA	BglJ-RcsB site mutant 2
T354	GTGTAATTTg <u>AG</u> GctATTTgaAAcGTTATATATAACAAATCCCAATAATTAAGTTA	BglJ-RcsB site mutant 3
T358	GACCGA <u>ATTC</u> TTGCTGTAGCAAGGTAGCCTATTACATG	rcsB 5' (EcoRI)
T359	<u>gaccgaattc</u> CACTCATAGAAAAATGCGTCATGAGTAGTAT	bglJ 5' (2 nd AUG, EcoRI)
T360	<u>gaccgaattc</u> AGGAGTCATTTCCAGGATGCCATGT	bglJ 5' (3 rd AUG, EcoRI)
T397	TGTTGATTACC <u>g</u> gCTCTCCATGCGCTGGCGATAAG	rcsB-D56A
T398	GCATGGAGAG <u>g</u> gCGGTAATCAACACATGCGCATCC	rcsB-D56A

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