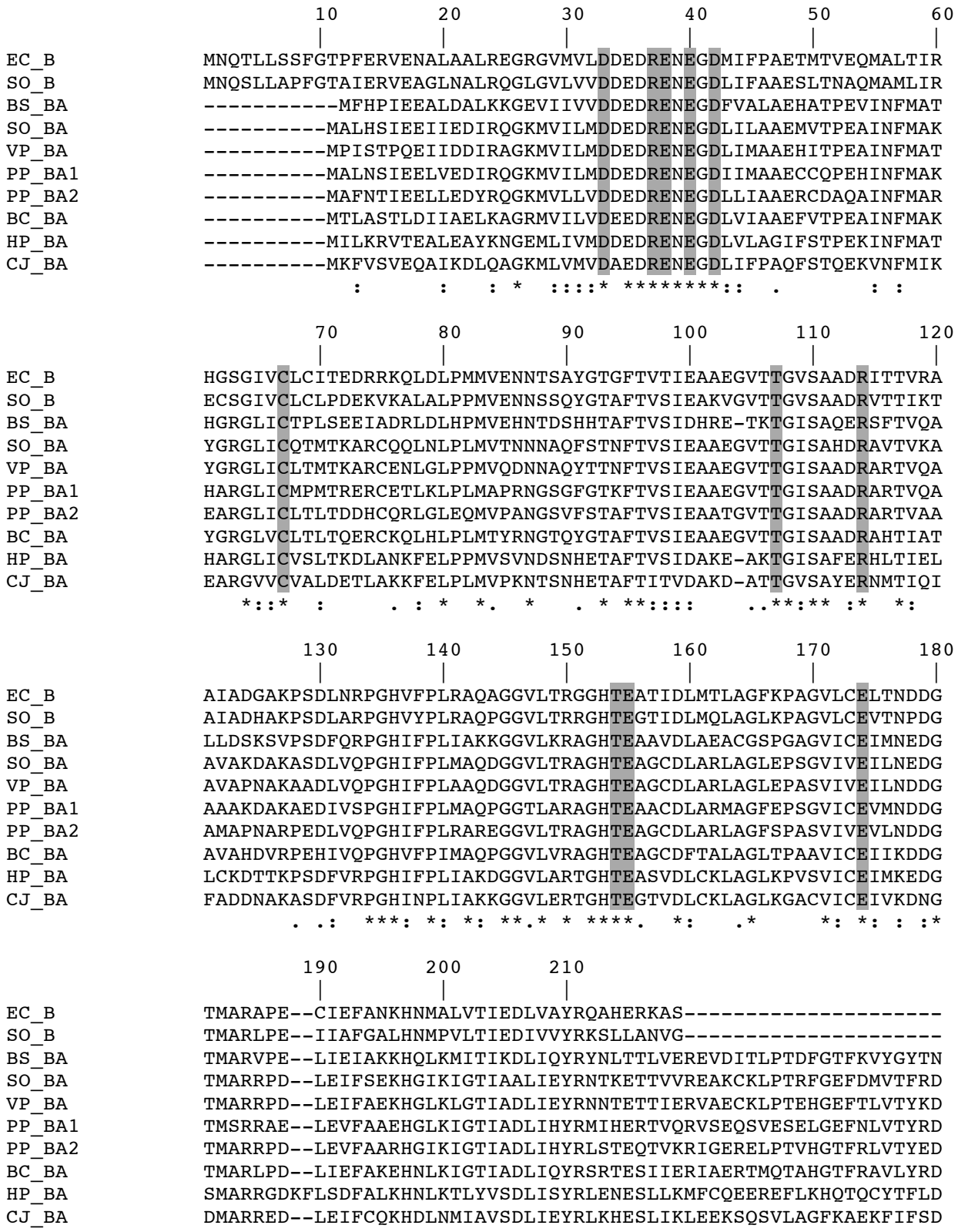
Figure S1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SO_B MNQSLLAPFGTAIERVEAGLNALRQGLGVLVVDDEDRENEGDLIFAAESLTNAQMAMLIR
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SO_BA ----------MALHSIEEIIEDIRQGKMVILMDDEDRENEGDLILAAEMVTPEAINFMAK
VP_BA ----------MPISTPQEIIDDIRAGKMVILMDDEDRENEGDLIMAAEHITPEAINFMAT
PP_BA1 ----------MALNSIEELVEDIRQGKMVILMDDEDRENEGDIIMAAECCQPEHINFMAK
PP_BA2 ----------MAFNTIEELLEDYRQGKMVLLVDDEDRENEGDLLIAAERCDAQAINFMAR
BC_BA ----------MTLASTLDIIAELKAGRMVILVDEEDRENEGDLVIAAEFVTPEAINFMAK
HP_BA ----------MILKRVTEALEAYKNGEMLIVMDDEDRENEGDLVLAGIFSTPEKINFMAT
CJ_BA ----------MKFVSVEQAIKDLQAGKMLVMVDAEDRENEGDLIFPAQFSTQEKVNFMIK
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ECSGIVCLCLPDEKVKALALPPMVENNSSQYGTAFTVSIEAKVGVTTGVSAADRVTTIKT
HGRGLICTPLSEEIADRLDLHPMVEHNTDSHHTAFTVSIDHRE-TKTGISAQERSFTVQA
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EARGVVCVALDETLAKKFELPLMVPKNTSNHETAFTITVDAKD-ATTGVSAYERNMTIQI
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FADDNAKASDFVRPGHINPLIAKKGGVLERTGHTEGTVDLCKLAGLKGACVICEIVKDNG
TMARAPE--CIEFANKHNMALVTIEDLVAYRQAHERKAS----------
TMARLPE--IIAFGALHNMPVLTIEDIVVYRKSLLANVG----------
TMARVPE--LIEIAKKHQLKMITIKDLIQYRYNLTTLVEREVDITLPTDFGTFKVYGYTN
TMARRPD--LEIFSEKHGIKIGTIAALIEYRNTKETTVVREAKCKLPTRFGEFDMVTFRD
TMARRPD--LEIFAEKHGLKLGTIADLIEYRNNTETTIERVAECKLPTEHGEFTLVTYKD
TMSRRAE--LEVFAAEHGLKIGTIADLIHYRMIHERTVQRVSEQSVESELGEFNLVTYRD
TMARRPD--LEVFAARHGIKIGTIADLIHYRLSTEQTVKRIGERELPTVHGTFRLVTYED
TMARLPD--LIEFAKEHNLKIGTIADLIQYRSRTESIIERIAERTMQTAHGTFRAVLYRD
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DMARRED--LEIFCQKHDLNMIAVSDLIEYRLKHESLIKLEEKSQSVLAGFKAEKFIFSD

Figure S1. Alignment of DHBP synthase domains from *Proteobacteria*. (A) ClustalW alignment of the *E. coli* and MR-1 RibB sequences and the RibBA-NTD sequences of select *Proteobacteria*. Based on the published crystal structure of the *E. coli* RibB (1), residues that participate in the catalytic mechanism are boxed in dark grey.

Reference:

1. Liao DI, Calabrese JC, Wawrzak Z, Viitanen PV, & Jordan DB (2001) Crystal structure of 3,4-dihydroxy-2-butanone 4-phosphate synthase of riboflavin biosynthesis. *Structure* 9(1):11-18.

Figure S2

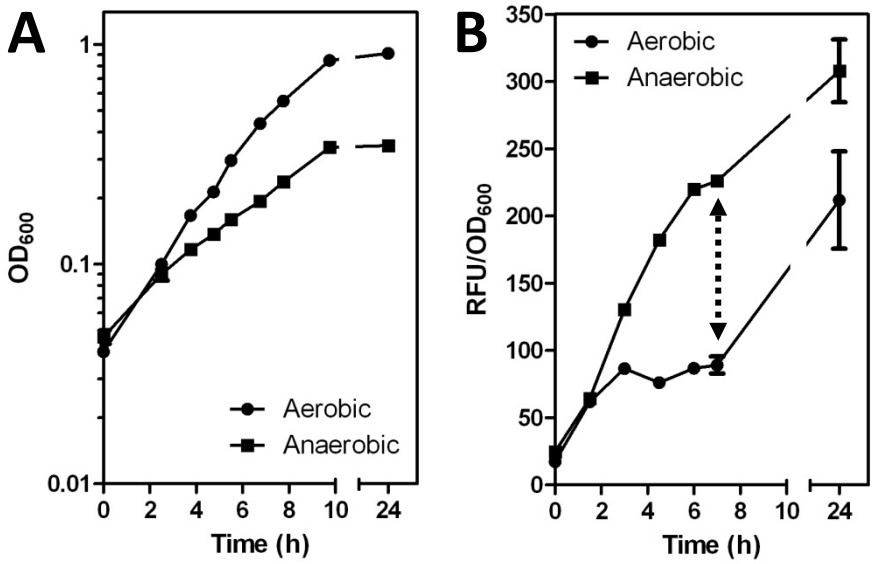

Figure S2. Growth of MR-1 and accumulation of extracellular flavins in the presence and absence of oxygen. (A) Optical density (OD_{600}) of wild-type MR-1 grown aerobically (●) or anaerobically (■) in SBM. (B) Fluorescence intensity (in RFU) normalized to optical density from culture supernatants of wild-type MR-1 grown aerobically (●) or anaerobically (■) in SBM. The time point used for Figure 2 is indicated with a dotted arrow. Reported values for both A and B are the averages of three independent experiments and error bars represent the standard error of the mean (SEM).

Table S1. Strains and plasmids used in this study[1].

Strain	Description	Source
Shewanella oneidensis		
MR-1 (JG274)	Wild-type MR-1	(*1*)
JG1604	Δ*ribB*	This work
JG1667	Δ*ribBX*	This work
JG2240	Δ*ribE-1*	This work
JG2241	Δ*ribE-2*	This work
JG168	MR-1 + pBBR1MCS-2	This work
JG1598	MR-1 + pBBR1MCS-2 *ribA*	This work
JG1599	MR-1 + pBBR1MCS-2 *ribB*	This work
JG1775	MR-1 + pBBR1MCS-2 *ribBX*	This work
JG1995	MR-1 + pBBR1MCS-2 $ribBA_{BS}$	This work
JG1818	MR-1 + pBBR1MCS-2 *ribBX-NTD*	This work
JG1819	MR-1 + pBBR1MCS-2 *ribBX-CTD*	This work
Escherichia coli		
UQ950	DH5α λpir cloning strain	(*2*)
WM3064	Donor strain for conjugation	(*2*)
1100-2	Parent strain of the riboflavin auxotrophs	(*3*)
BSV11	1100-2 *ribB11::Tn5*	(*3*)
BSV18	1100-2 *ribA18::Tn5*	(*3*)
BSV23	1100-2 *ribC23::Tn5* (*ribE::Tn5*)	(*3*)
JG1725	BSV11 + pBBR1MCS-5	This work
JG1908	BSV11 + pBBR1MCS-5 *ribA*	This work
JG1726	BSV11 + pBBR1MCS-5 *ribB*	This work
JG1727	BSV11 + pBBR1MCS-5 *ribBX*	This work
JG1909	BSV11 + pBBR1MCS-5 *ribBX-NTD*	This work
JG1910	BSV11 + pBBR1MCS-5 *ribBX-CTD*	This work
JG1997	BSV11 + pBBR1MCS-5 $ribBA_{BS}$	This work
JG2213	BSV11 + pBBR1MCS-5 $ribBA_{BS}$-*NTD*	This work
JG2214	BSV11 + pBBR1MCS-5 $ribBA_{BS}$-*CTD*	This work
JG2537	BSV11 + pBBR1MCS-5 VP_0618	This work
JG2538	BSV11 + pBBR1MCS-5 PP_0516	This work
JG2539	BSV11 + pBBR1MCS-5 PP_3813	This work
JG2540	BSV11 + pBBR1MCS-5 BcenP_01000846	This work
JG1722	BSV18 + pBBR1MCS-5	This work
JG1723	BSV18 + pBBR1MCS-5 *ribA*	This work
JG1911	BSV18 + pBBR1MCS-5 *ribB*	This work
JG1724	BSV18 + pBBR1MCS-5 *ribBX*	This work
JG1912	BSV18 + pBBR1MCS-5 *ribBX-NTD*	This work
JG1913	BSV18 + pBBR1MCS-5 *ribBX-CTD*	This work
JG1998	BSV18 + pBBR1MCS-5 $ribBA_{BS}$	This work
JG2215	BSV18 + pBBR1MCS-5 $ribBA_{BS}$-*NTD*	This work
JG2216	BSV18 + pBBR1MCS-5 $ribBA_{BS}$-*CTD*	This work
JG2543	BSV18 + pBBR1MCS-5 VP_0618	This work
JG2544	BSV18 + pBBR1MCS-5 PP_0516	This work

JG2545	BSV18 + pBBR1MCS-5 PP_3813	This work
JG2546	BSV18 + pBBR1MCS-5 BcenP_01000846	This work
JG1905	BSV23 + pBBR1MCS-5	This work
JG1903	BSV23 + pBBR1MCS-5 *ribE-1*	This work
JG1904	BSV23 + pBBR1MCS-5 *ribE-2*	This work
SU101	Mono-hybrid *lacZ* reporter stain	(*4*)
SU202	Two-hybrid *lacZ* reporter stain	(*4*)
SU101 Vec	SU101 + pSR658	(*4*)
SU101 LexA-CAT	SU101 + pDD506	(*4*)
JG2414	SU101 + pSR658 *ribA*	This work
JG2415	SU101 + pSR658 *ribB*	This work
JG2416	SU101 + pSR658 *ribBX*	This work
JG2417	SU101 + pSR658 *ribBX-NTD*	This work
JG2418	SU101 + pSR658 *ribBX-CTD*	This work
JG2419	SU101 + pSR658 $ribBA_{BS}$	This work
SU202 Vec1 Vec2	SU202 + pSR658 and pSR659	(*4*)
SU202 Jun/Fos	SU202 + pMS604 and pDP804	(*4*)
JG2420	SU202 + pSR658 *ribBX-CTD* and pSR659	This work
JG2421	SU202 + pSR658 *ribBX-CTD* and pSR659 *ribA*	This work
JG2422	SU202 + pSR658 *ribBX-CTD* and pSR659 *ribB*	This work
JG2423	SU202 + pSR658 *ribBX-CTD* and pSR659 *ribBX*	This work
JG2424	SU202 + pSR658 *ribBX-CTD* and pSR659 *ribBX-NTD*	This work
JG2425	SU202 + pSR658 *ribBX-CTD* and pSR659 *ribBX-CTD*	This work

Plasmid	**Description**	**Source**
pSMV3 *ribA*	Deletion vector for *ribA*	This work
pSMV3 *ribB*	Deletion vector for *ribB*	This work
pSMV3 *ribBX*	Deletion vector for *ribBX*	This work
pSMV3 *ribE-1*	Deletion vector for *ribE-1*	This work
pSMV3 *ribE-2*	Deletion vector for *ribE-1*	This work
pBBR1MCS-2	Broad host range vector, Km	(*5*)
pBBR1MCS-2 *ribA*	*ribA* from MR-1	This work
pBBR1MCS-2 *ribB*	*ribB* from MR-1	This work
pBBR1MCS-2 *ribBX*	*ribBX* from MR-1	This work
pBBR1MCS-2 $ribBA_{BS}$	*ribBA* from *Bacillus subtilis subtilis* 168	This work
pBBR1MCS-2 *ribBX-NTD*	nt 1 - 621 of *ribBX* from MR-1	This work
pBBR1MCS-2 *ribBX-CTD*	nt 540 - 1101 of *ribBX* from MR-1	This work
pBBR1MCS-5	Broad host range vector, Gm	(*5*)
pBBR1MCS-5 *ribA*	*ribA* from MR-1	This work
pBBR1MCS-5 *ribB*	*ribB* from MR-1	This work
pBBR1MCS-5 *ribBX*	*ribBX* from MR-1	This work
pBBR1MCS-5 *ribBX-NTD*	nt 1 - 621 of *ribBX* from MR-1	This work
pBBR1MCS-5 *ribBX-CTD*	nt 540 - 1101 of *ribBX* from MR-1	This work
pBBR1MCS-5 $ribBA_{BS}$	*ribBA* from *B. subtilis subtilis* 168	This work
pBBR1MCS-5 $ribBA_{BS}$-*NTD*	*ribBA* from *B. subtilis subtilis* 168	This work
pBBR1MCS-5 $ribBA_{BS}$-*CTD*	*ribBA* from *B. subtilis subtilis* 168	This work
pBBR1MCS-5 *ribE-1*	*ribE-1* from MR-1	This work
pBBR1MCS-5 *ribE-2*	*ribE-2* from MR-1	This work

pBBR1MCS-5 VP_0618	VP_0618 from *Vibrio parahaemolyticus* RIMD	This work
pBBR1MCS-5 PP_0516	PP_0516 from *Pseudomonas putida* KT2440	This work
pBBR1MCS-5 PP_3813	PP_3813 from *P. putida* KT2440	This work
pBBR1MCS-5 BcenP_01000846	PP_0516 from *Burkholderia cenocepacia* PC184	This work
pSR658	LexA fusions for mono- and two-hybrid	(*4*)
pSR659	LexA fusions for two-hybrid	(*4*)
pDD506	pSR658 expressing the CAT-LexA fusion	(*4*)
pMS604	pSR658 expressing the Jun-LexA fusion	(*4*)
pDP804	pSR659 expressing the Fos-LexA fusion	(*4*)
pSR658 *ribA*	*lexA-ribA* from MR-1	This work
pSR658 *ribB*	*lexA-ribB* from MR-1	This work
pSR658 *ribBX*	*lexA-ribBX* from MR-1	This work
pSR658 *ribBX-NTD*	*lexA-ribBX-NTD* from MR-1	This work
pSR658 *ribBX-CTD*	*lexA-ribBX-CTD* from MR-1	This work
pSR658 $ribBA_{BS}$	*lexA-*$ribBA_{BS}$ from MR-1	This work
pSR659 *ribA*	*lexA-ribA* from MR-1	This work
pSR659 *ribB*	*lexA-ribB* from MR-1	This work
pSR659 *ribBX*	*lexA-ribBX* from MR-1	This work
pSR659 *ribBX-NTD*	*lexA-ribBX-NTD* from MR-1	This work
pSR659 *ribBX-CTD*	*lexA-ribBX-CTD* from MR-1	This work

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[1]To avoid confusion the gene in MR-1 formerly named *ribBA* (SO_3467) is referred to by the new name *ribBX* throughout this table.

1. C. R. Myers, K. H. Nealson, Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science* **240**, 1319 (Jun, 1988).
2. C. W. Saltikov, D. K. Newman, Genetic identification of a respiratory arsenate reductase. *Proc Natl Acad Sci U S A* **100**, 10983 (Sep, 2003).
3. S. V. Bandrin, P. M. Rabinovich, A. I. Stepanov, [3 linkage groups of the genes of riboflavin biosynthesis in *Escherichia coli*]. *Genetika* **19**, 1419 (Sep, 1983).
4. D. A. Daines, M. Granger-Schnarr, M. Dimitrova, R. P. Silver, Use of LexA-based system to identify protein-protein interactions in vivo. *Methods Enzymol* **358**, 153 (2002).
5. M. E. Kovach *et al.*, Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**, 175 (Dec, 1995).

Table S2. Oligonucleotide primers used in this study.

Primer	Sequence*
Deletion primer sets for cloning up and downstream flanking regions into pSMV3	
ribA Upstream Forward	gcactcGGATCCATCACTCTCTGGCGGCGATCC
ribA Upstream Reverse	gcactcCTCGAGCGACATGACTTAACCTTAACGAC
ribA Downstream Forward	gcactcCTCGAGGAAGAGTAGTTTCAAGGCAATATATTTACCG
ribA Downstream Reverse	gcactcGAGCTCCTAACATTAAGCTGCACACTAAATAGGG
ribB Upstream Forward	gcactcGGATCCTATCGGCGGTAATAGCTATGCTGATGCC
ribB Upstream Reverse	gcactcCTCGAGCTGATTCATGGTTAATATCCTTCAAAAATACGACATGGG
ribB Downstream Forward	gcactcCTCGAGGGCTAAATTTGGCCCCATAAACGGG
ribB Downstream Reverse	gcactcGAGCTCTAATGCTGAGGCTATCTGACAACAACATACG
ribBX Upstream Forward	gcactcGGATCCGCTATTTAGGCAAAAGCTGTAATCAGGTGC
ribBX Upstream Reverse	gcactcACTAGTCGCCATTGTAGGACCTTTATAATGTGACTGTTG
ribBX Downstream Forward	gcactcACTAGTGCTGAGTAACAGGTTAAGCTTGTAATAGGTTGCAC
ribBX Downstream Reverse	gcactcGAGCTCAACAAACTTATGGCTTTCATCCGCACCGAAAGC
ribE-1 UpstreamForward	gcatGGATCCACAGGGCTAACAATAAGCCG
ribE-1 UpstreamReverse	gcatACTAGTAGTAAACATTTTTTGCTCGCTTATTCG
ribE-1 Downstream Forward	gcatACTAGTCTATCTTAATAAAAAGCCTAGTAAATTTGC
ribE-1 Downstream Reverse	gcatGCGGCCGCCCACACGTAGGGCCACATGGTG
ribE-2 UpstreamForward	gcatGGATCCGAGCCCCACGCTGAAGTGCATGCGC
ribE-2 UpstreamReverse	gcatACTAGTAGTAAACATGGTTAACTCACTTATTCGC
ribE-2 Downstream Forward	gcatACTAGTGTGCGTTAGTACACTGGCACTC
ribE-2 Downstream Reverse	gcatGAGCTCCTTGATCTTCCGCTTCAAAGGC
Complementation primer sets for cloning the indicated gene into pBBR1MCS-2 and / or pBBR1MCS-5	
ribA Forward	gcactcGAATTCTGGAATGAGAGTTGTCGTTAAGG
ribA Revese	gcactcGGATCCCTACTCTTCATCCGTAAAATGATAC
ribB Forward	gcactcGAATTCCCCATGTCGTATTTTTGAAGGATATTAACC
ribB Revese	gcactcGGATCCTTAGCCTACGTTGGCCAATAAGGATTTACGG
ribBX Forward	gcactcGAATTCCATAACAACAGTCACATTATAAAGGTCC
ribBX Reverse	gcactcGGATCCTTACTCAGCTACGCATTCGGTCACTTCAAGGCC
ribBX-NTD Forward	gcactcGAATTCCATAACAACAGTCACATTATAAAGGTCC
ribBX-NTD Reverse	gcactcGGATCCTTACACAACGGTCGTTTCTTTGGTATTACGG
ribBX-CTD Forward	gcactcGAATTCCATAACAACAGTCACATTATAAAGGTCCTACAATGAAAATCGGCACTATTGCGGCCCTG
ribBX-CTD Reverse	gcactcGGATCCTTACTCAGCTACGCATTCGGTCACTTCAAGGCC
ribBA$_{BS}$ Forward	gcatGAATTCCATAACAACAGTCACATTATAAAGGTCCTACAATGTTTCATCCGATAGAAGAAG CACTGG
ribBA$_{BS}$ Reverse	gcatGGATCCTTAGAAATGAAGTAAATGACCTAGC
ribBA$_{BS}$-NTD Forward	gcatGAATTCCATAACAACAGTCACATTATAAAGGTCCTACAATGTTTCATCCGATAGAAGAAGCACTGG
ribBA$_{BS}$-NTD Reverse	gcatGGATCCTTACTCGACAAGTGTTGTCAGATTGTAACGG
ribBA$_{BS}$-CTD Forward	gcatGAATTCCATAACAACAGTCACATTATAAAGGTCCTACAATGATCACCATTAAGGATTTGATTC
ribBA$_{BS}$-CTD Reverse	gcatGGATCCTTAGAAATGAAGTAAATGACCTAGC
ribE-1 Forward	gcactcGAATTCCAGCCTTTAACGAATAAGCGAGC
ribE-1 Reverse	gcactcGGATCCTTAAGATAGTCCGTTAAAACGTTTAGCC
ribE-2 Forward	gcactcGAATTCCCATCTTTAGCGAATAAGTGAGTTAACC
ribE-2 Reverse	gcactcGGATCCCTAACGCACAAAGCCAGCACGGGC
VP_0618 Forward	gcacGAATTCCACATTATAAAGGTCCTACAATGCCAATCAGTACACCACAAG
VP_0681 Reverse	gcacGGATCCTTATTCGCAAACGTATTCAACCACG
PP_0516 Forward	gcacGAATTCCACATTATAAAGGTCCTACAATGGCGCTCAACAGCATCGAAGAAC
PP_0516 Reverse	gcacGGATCCTCACTCGGAGGGCACGTATTCTAC
PP3813 Forward	gcacGAATTCCACATTATAAAGGTCCTACAATGGCTTTCAACACCATCGAGGAAC
PP3813 Reverse	gcacGGATCCTCAGCCATCCATCCCCGGGTCGAACG

BcenP_01000846 Forward	gcacGGATCCCACATTATAAAGGTCCTACAATGACGCTCGCCTCCACGCTCGAC
BcenP_01000846 Reverse	gcacGAGCTCTTACGCGTGGGACGCCGGGCAGGAC

Primer sets for cloning the indicated gene as a *lexA* fusion in pSR658 and / or pSR659

ribA Forward	gcatCTCGAGATGTCGATAAAATATGTCGCG
ribA Reverse	gcatGGTACCCTACTCTTCATCCGTAAAATG
ribB Forward	gcatCTCGAGATGAATCAGTCTTTACTTGCTCC
ribB Reverse	gcatGGTACCTTAGCCTACGTTGGCCAATAAGG
ribBX Forward	gcatCTCGAGATGGCGCTGCACAGTATAGAAG
ribBX Reverse	gcatGGTACCTTACTCAGCTACGCATTCGGTC
ribBX-NTD Forward	gcatCTCGAGATGGCGCTGCACAGTATAGAAG
ribBX-NTD Reverse	gcatGGTACCTTACACAACGGTCGTTTCTTTGG
ribBX-CTD Forward	gcatCTCGAGATGAAAATCGGCACTATTGCGG
ribBX-CTD Reverse	gcatGGTACCTTACTCAGCTACGCATTCGGTC
$ribBA_{BS}$ Forward	gcatCTCGAGATGTTTCATCCGATAGAAGAAGCACTGG
$ribBA_{BS}$ Reverse	gcatGGTACCTTAGAAATGAAGTAAATGACCTAGC

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* - Restriction sites added by the oligonucleotide primer are underlined and nucleotides added to the 5' end to facilitate subsequent restriction digests are in lower case lettering. Nucleotides boxed in light grey are the *ribBX* leader region added by the oligonucleotide primer to facilitate translation. Nucleotides boxed in dark grey are start or stop codons that were added when necessary.