Table 3. Plasmid details

p*ralF*	1179ON2 1179C3	G**GAATTC**GAGCTCAGACTAAAGGAGCAGATTATG GC**TCTAGA**TCAAACTATCGAGGCCATTTC	pMMB207	*Eco*RI *Xba*I
pSB-*ralF*-M45	1179ON2 1179OC	G**GAATTC**GAGCTCAGACTAAAGGAGCAGATTATG CATG**CCATGG**AAAATTTTAATTGTCTACCTTTTTC	pSB616	*Eco*RI *Nco*I*
p*ralF*-M45	Subcloned from pSB-*ralF*-M45		pMMB207	*Eco*RI *Xba*I
pM45-*ralF*	1179ON 1179C3	CG**GGATCC**TTATGCATCCAGAAATTGAAAAAG GC**TCTAGA**TCAAACTATCGAGGCCATTTC	pMMB207-N45NT	*Bam*HI *Xba*I
p*cya-ralF*	CyaNN CyaNCNotI	GA**AGATCT**TACAGCAATCGCATCAGGCTGG CG**GGATCC**GCGGCCGCCTGTCATAGCCGGAATCCTGG	pM45-*ralF*	*Bgl*II *Bam*HI[†,‡]
p*cya*	CyaNN CyaNCfs	GA**AGATCT**TACAGCAATCGCATCAGGCTGG CG**GGATCC**CTGTCATAGCCGGAATCCTGG	pM45-*ralF*	*Bgl*II *Bam*HI[†,‡,§]
p*ralF*$_{1-305}$-*cya*	ClaXbaCya CyaPst	GC**ATCGAT**TCTAGAATGCAGCAATCGCATCAGGC GCG**CTGCAG**TTAGTCATAGCCGGAATCCTGGC	pM45-*ralF*	*Cla*I *Pst*I*[,†]
p*ralF-cya*	Seq1485 RalFXbaNew	GGACAAACCAAAGAGTCA**ATCG** GC**TCTAGA**AAATTTTAATTGTCTACCTTTTTC	p*ralF*$_{1-305}$-*cya*	*Cla*I *Xba*I
p*ralF*$_{1-194}$	1174ON2 S7-194r	G**GAATTC**GAGCTCAGACTAAAGGAGCAGATTATG CCGAGC**TCTAGA**TTACAACTCAAAAGGTTTGGCTTTAATC	pMMB207	*Eco*RI *Xba*I*
p*cya-ralF*$_{1-194}$	1179ON1 pMMB2073	CG**GGATCC**TTATGCATCCAGAAATTGAAAAAG CAGACCGCTTCTGCGTTCTG	p*cya-ralF*	*Bam*HI *Pst*I[¶]
p*ralF*$_{1-339}$	1179ON2 ralF+340	G**GAATTC**GAGCTCAGACTAAAGGAGCAGATTATG GATC**GTCGAC**TTAGTTATCATATGCTTTTATTAAATC	pMMB207	*Eco*RI *Sal*I
p*cya-ralF*$_{1-339}$	1179ON1 pMMB2073	CG**GGATCC**TTATGCATCCAGAAATTGAAAAAG CAGACCGCTTCTGCGTTCTG	p*cya-ralF*	*Bam*HI *Pst*I[¶]
	Seq1485	GGACAAACCAAAGAGTCA**ATCG**	p*cya-ralF*	*Cla*I *Pst*I

	and			
p*cya-ralF*$_{1\text{-}344}$	345C	GCG**CTGCAG**TTAAATGAGTTTTTTCTGGTTATC		
p*cya-ralF*$_{1\text{-}349}$	350C	GCG**CTGCAG**TTAGTTTCTCTCAATCGTAATGAG		
p*cya-ralF*$_{1\text{-}354}$	355C	GCG**CTGCAG**TTACTCCTTAAGTGCCAGGTTTC		
p*cya-ralF*$_{1\text{-}359}$	360C	GCG**CTGCAG**TTAATCTTTGGGAACGCCCTCC		
p*cya-ralF*$_{1\text{-}364}$	365C	GCG**CTGCAG**TTACATTTCAGCGTCTGGATCTTTG		
p*cya-ralF*$_{1\text{-}369}$	370C	GCG**CTGCAG**TTAACCTTTTTCTTTTTGCATTTC		
p*cya-ralF*$_{1\text{-}370}$	371C	GCG**CTGCAG**TTATCTACCTTTTTCTTTTTGCATTTC		
p*cya-ralF*$_{1\text{-}371}$	372C	GCG**CTGCAG**TTATTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{1\text{-}372}$	373C	GCG**CTGCAG**TTATAATTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{1\text{-}373}$	374C	GCG**CTGCAG**TTATTTTAATTGTCTACCTTTTTC		
p*cya-ralF*$_{16\text{-}374}$	16N	CG**GGATCC**TTTTCAATGCCAAGCCAAAAAATG	p*cya-ralF*	*Bam*HI *Pst*I
p*cya-ralF*$_{222\text{-}374}$	222N	CG**GGATCC**TTTTCTTGCATTCAACGGATGTAAAC		
p*cya-ralF*$_{301\text{-}374}$	301N	CG**GGATCC**TTACCAAAGAGTCAATCGATTTG		
p*cya-ralF*$_{340\text{-}374}$	340N	CG**GGATCC**TTCAGAAAAAACTCATTACGATTG		
p*cya-ralF*$_{345\text{-}374}$	345N	CG**GGATCC**TTACGATTGAGAGAAACCTGGC		
p*cya-ralF*$_{350\text{-}374}$	350N	CG**GGATCC**TTCTGGCACTTAAGGAGGGCG		
p*cya-ralF*$_{355\text{-}374}$	355N	CG**GGATCC**TTGGCGTTCCCAAAGATCCAGAC		
p*cya-ralF*$_{360\text{-}374}$	360N	CG**GGATCC**TTCCAGACGCTGAAATGCAAAAAG		
p*cya-ralF*$_{365\text{-}374}$	365N	CG**GGATCC**TTCAAAAAGAAAAAGGTAGAC		
p*cya-ralF*$_{370\text{-}374}$	370N	CG**GGATCC**TTAGACAATTAAAATTTTAATCC		
	and			
	RalFC4	GC**GCTGCA**GTCTAGACAAACTATCGAGGCCATTTC		
	Seq1485	GGACAAACCAAAGAGTCA**ATCG**	p*cya-ralF*	*Cla*I *Pst*I
	and			
p*cya-ralF*$_{L372V}$	372V	GCG**CTGCAG**TTAAAATTTTACTTGTCTACCTTTTTCTTTTTG		

p*cya-ralF*$_{L372F}$	372F	GCG**CTGCAG**TTAAAATTTAAATTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{L372P}$	372P	GCG**CTGCAG**TTAAAATTTTGGTTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{L372S}$	372S	GCG**CTGCAG**TTAAAATTTTGATTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{L372T}$	372T	GCG**CTGCAG**TTAAAATTTTGTTTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{L372A}$	372A	GCG**CTGCAG**TTAAAATTTTGCTTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{K373E}$	373E	GCG**CTGCAG**TTAAAATTCTAATTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{K373A}$	373A	GCG**CTGCAG**TTAAAATGCTAATTGTCTACCTTTTTCTTTTTG		
p*cya-ralF*$_{K373R}$	373R	GCG**CTGCAG**TTAAAATCTTAATTGTCTACCTTTTTCTTTTTG		
pJV450-*cya-ralF*	Subcloned from p*cya-ralF*		pJV450	*Eco*RI *Sac*I/*Pst*I (blunted)

Forward primers are listed first followed by the reverse primer. Restriction enzyme sites used in plasmid construction are bold.

*These plasmids represent intermediate products and were not used in the experiments described in the text.

†pMS107 was used as a PCR template.

‡PCR fragments were digested with *Bgl*II and *Bam*HI. The resulting fragments were ligated into *Bam*HI-digested pM45-ralF. The resulting plasmids in the right orientation have unique *Bam*HI sites at the junction of the *cya* and the *ralF* genes.

§p*cya* has a frameshift at the junction of the *cya* and the *ralF* genes. The resulting gene expresses a Cya protein with an 8-aa extension on the C terminus as shown in Fig. 1*A*.

¶‖p*ralF*$_{1-194}$ and p*ralF*$_{1-339}$ was used as a PCR template, respectively. The reverse primer (pMMB2073) anneals to DNA downstream of the cloning sites in these plasmids.