

Supplementary Table S4: Plasmid construction.

1st PCRs	Matrix	2 nd PCR	Digestion	Vector	Analysis
Construction of pGΩΔ $abx1$ for $abx1$ in frame deletion					
a) KO_ $abx1$ _E + KO_ $abx1$ _rv	gDNA NEM316	KO_ $abx1$ _E + KO_ $abx1$ _B	EcoRI BamHI	pG ⁺ host5	KO_ $abx1$ _5 KO_ $abx1$ _3
b) KO_ $abx1$ _fw + KO_ $abx1$ _B	gDNA NEM316				
Construction of pGΩΔ $covR$ for $covR$ in frame deletion					
a) O1 + O2	gDNA NEM316	O1 + O4	EcoRI	pG ⁺ host5	
b) O3 + O4	gDNA NEM316		BamHI		
Construction of pGΩΔ $covS$ for $covS$ in frame deletion					
a) O5 + O6	gDNA NEM316	O5 + O8	KpnI	pG ⁺ host5	
b) O7 + O8	gDNA NEM316		BamHI		
Construction of pGΩΔ $stk1$ for $stk1$ in frame deletion					
a) Stk5 + Stk int1	gDNA NEM316		EcoRI	pG ⁺ host5	pAF174
b) Stk int2 + Stk3	gDNA NEM316		PstI		pAF175
Construction of pGΩΔP $abx1$::P _{cyl+} for $abx1$ promoter substitution					
a) KO_ $abx1$ _E + pAF152	gDNA NEM316	KO_ $abx1$ _E + pAF151	EcoRI	pG ⁺ host5	
b) pAF153 + pAF151	pTCVΩP _{cyl+} $abx1$	pAF151	BamHI		
Construction of pGΩ $cylE$ C ₆₆₄ A for CylE C ₆₆₄ A alanine substitution					
a) cylEA_E + cylEA_rev	gDNA NEM316	cylEA_E + cylEA_B	EcoRI BamHI	pG ⁺ host5	cylEA_5 cylEA_3
b) cylEA_fw + cylEA_B	gDNA NEM316				
Construction of pGΩ $covR$ D ₅₃ A for CovR D ₅₃ A alanine substitution					
a) O9 + O10	gDNA NEM316	O9 + O12	EcoRI	pG ⁺ host5	
b) O11 + O12	gDNA NEM316		BamHI		
Construction of pGΩ $covS$ H ₂₇₈ A for CovS H ₂₇₈ A alanine substitution					
a) pAF207 + pAF208	gDNA NEM316	pAF207 + pAF210	EcoRI	pG ⁺ host5	pAF50
b) pAF209 + pAF210	gDNA NEM316	pAF210	BamHI		pAF211
Construction of pGΩ $covS$ T ₂₈₂ A for CovS T ₂₈₂ A alanine substitution					
a) pAF207 + pAF407	gDNA NEM316	pAF207 + pAF210	EcoRI	pG ⁺ host5	pAF50
b) pAF408 + pAF210	gDNA NEM316	pAF210	BamHI		pAF211
Construction of pTCVΩ $abx1$ complementing vector					
pAF18 + pAF19	gDNA NEM316		EcoRI XbaI	pTCV-erm	
Construction of pTCVΩP _{cyl+} $abx1$ overexpression vector					
a) pAF52 + pAF53	gDNA CCH206		EcoRI	pTCV-erm	
b) pAF54 + pAF19	gDNA NEM316		XbaI		
Construction of pTCVΩP _{tet} $abx1$ overexpression vector					
a) pAF206 + pAF149	pTCV_P _{tet}	pAF206 + 1532_B	EcoRI	pTCV-erm	
b) pAF150 + 1532_B	gDNA NEM316	1532_B	BamHI		
Construction of pTCVΩP _{cyl+} $gbs1037$ overexpression vector					
a) pAF52 + pAF203	gDNA CCH206	pAF52 + pAF205	EcoRI	pTCV-erm	
b) pAF204 + pAF205	gDNA NEM316	pAF205	BamHI		
Construction of pTCVΩP _{cyl+} $EGFP$ overexpression vector					
a) pAF52 + pAF200	gDNA CCH206	pAF52 + pAF202	EcoRI	pTCV-erm	
b) pAF201 + pAF202	EGFP	pAF202	BamHI		
Construction of pTCVΩ $abx1$ -(Ala) alanine substitution vector					
a) pAF18 + X-rev	pTCVΩ $abx1$	pAF18 + 1532_B	EcoRI	pTCV-erm	
b) X-fw + 1532_B	pTCVΩ $abx1$	1532_B	BamHI		
Construction of pTCVΩ stp _ stk complementing vector					
pAF69 + pAF70	gDNA NEM316		XbaI	pTCV-erm	pAF174 pAF306

Construction of fusion proteins for double hybrid

Cloning in pKNT25 and pUT18 (T18 / T25 tags at the C-terminal of the fusion proteins)

Abx1 = pAF216 + pAF217	gDNA NEM316	HindIII EcoRI	pAF230
CovS = pAF218 + pAF219		PstI BamHI	pAF231
CovR = pAF220 + pAF221		HindIII EcoRI	pAF286
Gbs2082 = pAF239 + pAF240		PstI BamHI	
Gbs0430 = pAF245 + pAF246		PstI BamHI	
CovS_form I = pAF236 + pAF219		PstI BamHI	
CovS form II = pAF305 + pAF219		PstI BamHI	
CovS form III = pAF305 + pAF235		PstI BamHI	

Cloning in pKT25 and pUT18C (T18 / T25 tags at the N-terminal of the fusion proteins)

Abx1 = pAF443 + pAF444	gDNA NEM316	BamHI KpnI	pAF208
CovS form IV = pAF500 + pAF517		BamHI KpnI	pAF209
CovS form V = pAF500 + pAF516		BamHI KpnI	pAF210
CovS form VI = (pAF500 + pAF501) + (pAF502 + pAF503)		BamHI KpnI	pAF211
CovS form VII = pAF500 + pAF503		BamHI KpnI	
