Crosstalk Inhibition Nullified by a Receiver Domain Missense Substitution

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RESPONSE REGULATOR SEQUENCE FAMILIES

Receiver domain phylogenetic analysis was conducted as described in Materials and Methods. Sequences included in these analyses are listed in **Table S1**. A representative result is presented in **Fig. S1**; trees generated from different sequence subsets and/or with different parameters differed in detail but shared similar overall topologies with respect to the major groups (clades).

Results extend conclusions drawn from prior studies (1, 7, 8, 96, 97). We sort these response regulator sequences into four groups, based on receiver domain phylogenetic affinity and conserved sequence features as well as on output domains. Note that several receiver domain features differentiate the FixJ-StyR and NarL-VraR families.

OmpR-PhoB sequence family. These response regulators mostly have the winged helix DNA binding domain. Most interact with the closely-related HPK1, HpK2 or HPK3 sequence family transmitter modules (8), excepting a few that interact with Hpt domains from phosphorelays (see below).

NtrC-DctD receiver sequence family. These response regulators have a central ATPase domain plus the Fis DNA-binding domain, and interact with the distinct HPK4 sequence family of transmitter modules (8). Receiver domain sequences are similar to those of the OmpR-PhoB family; the most obvious distinction is at CheY position 104, which is Asp or Asn in the OmpR-PhoB family but Phe or Tyr in the NtrC-DctD family (**Fig. S2**).

FixJ-StyR sequence family. These response regulators have the GerE DNA binding domain. Nevertheless, the receiver domain sequence families clearly are distinct from those of the NarL-VraR family, both in phylogenetic position (**Fig. S1**) and in conserved sequence features (**Fig. S2**). Instead, the receiver domain sequences are very similar to those of the NtrC-DctD family, as noted previously (8, 96).

NarL-VraR sequence family. These response regulators all have the GerE DNA binding domain. Our analysis consciously included more representatives from this group, in part to explore sequence diversity at position T+1 (see below). Two sets of

sequences, represented by FlhR and BvgA, clustered on the edges of the NarL phylogenetic group (**Fig. S1**); however, these did not have obvious differentiating features.

Exceptional sequences. A few proteins included in this analysis have OmpR-PhoB family receiver domain sequences and interact with HPK1-type transmitters, and evidently arose through domain shuffling (8, 96). These are AlgB.Pa, which has a central ATPase domain plus the Fis DNA-binding domain characteristic of NtrC-DctD family members, and PprB.Pa, Pden_3311, and SMb20610, all of which a GerE family DNA binding domain. Interestingly, these latter three proteins have longer interdomain linker sequences (PprB.Pa, 73 residues; Pden_3311, 129; SMb20610, 114) than is typical for NarL-VraR or FixJ-StyR family members (13-36 residues).

RECEIVER DOMAIN SEQUENCE FEATURES

Fig. S2 shows sequence logos compiled from the receiver domain sequences used to construct **Fig. S1**. Note that sequences from the OmpR-PhoB, NtrC-DctD and FixJ-StyR families are quite similar to each other, so distinctions between these three families are based mostly on their different output domains. The NarL-VraR family sequences by contrast are quite distinct. Here we focus on three positions where conserved differences may have functional consequences.

Position T+1 (CheY residue 88). As noted previously by Volz (7), the NarL-VraR family exhibits considerable sequence diversity at this position, whereas most sequences from the other families have either Gly or Ala (**Fig. S2; Table S1**). The outer ring in **Fig. S1** is colored to indicate the identity of the T+1 residue for each sequence. It is immediately apparent that diversity at this position is scattered throughout the NarL-VraR family. Thus, the identity of the T+1 residue has no ostensible value in differentiating subgroups within this family.

Position K+1 (CheY residue 110). Again as noted previously by Volz (7), the NarL-VraR family exhibits considerable sequence diversity at this position, whereas most sequences from the other families have Pro (**Fig. S2; Table S1**). In the HK853-RR468 complex, receiver residue Pro-106 (K+1) contacts DHp residues Leu-266, Thr-267 and Lys-270, just carboxyl-terminal to the active site region spanning residues His-260 (phospho-accepting) through Thr-264 (81). The corresponding region in HisKA_3 sensors displays a different sequence conservation pattern (14). Thus, the absence of a Pro residue at position K+1 may reflect differences in detail for receiver domain interaction between HisKA and HisKA_3 DHp domains. indeed, a Glu-to-Lys missense

substitution at this position in NarL (E110K) apparently causes decreased interaction with the cross-regulating NarQ sensor (16).

Position DD+1 (CheY residue 14). The NarL-VraR family sequences have a polar His or Gln residue at this position, whereas most sequences from the other families have an acidic residue (Asp or Glu) (**Fig. S2; Table S1**). A His-to-Leu missense substitution at this position in DegU (Q12L) apparently causes decreased interaction with the cognate DegS sensor (99, 100), congruent with the recessive null phenotype conferred by the analogous substitution (H15L) in NarL (16).

ASSOCIATIONS WITH HYBRID SENSORS

Phosphorelays involve His-Asp-His-Asp phosphoryl transfer from the DHp domain (H1) through receiver (D1) and Hpt (H2) domains to the response regulator receiver domain (D2) (9). Tripartite hybrid sensors contain the H1, D1 and H2 domains in a single protein, whereas bipartite hybrid sensors contain only H1 and D1 domains, and transfer the phosphoryl group to a separate Hpt (H2) protein. **Fig. S1** indicates response regulators that partner with bipartite (green lines) and tripartite (red lines) sensors. For the subset of response regulators included in this analysis, those partnered with bipartite hybrid sensors cluster at one end of the NarL-VraR family (FlhR group). However, other bipartite hybrid sensors partner with response regulators from other families; one example is the LuxN sensor, which partners through the LuxU Hpt domain protein to the LuxO response regulator, a member of the NtrC-DctD family (Ng and Bassler, 2009, Annu. Rev. Genet. **43:**197–222).

Similarly, tripartite hybrid sensors partner with response regulators from different families; examples shown in **Fig. S1** include ArcB (partnered with the OmpR-PhoB family response regulator ArcA), and BarA (partnered with the NarL-VraR family response regulator UvrY). Thus, although there are strong associations between receiver domain and transmitter sequence families for standard two-component pairs, there is no obvious association for phosphorelays. This may reflect distinct specificity determinants for different Hpt domain families, which to our knowledge have not yet been defined in detail.

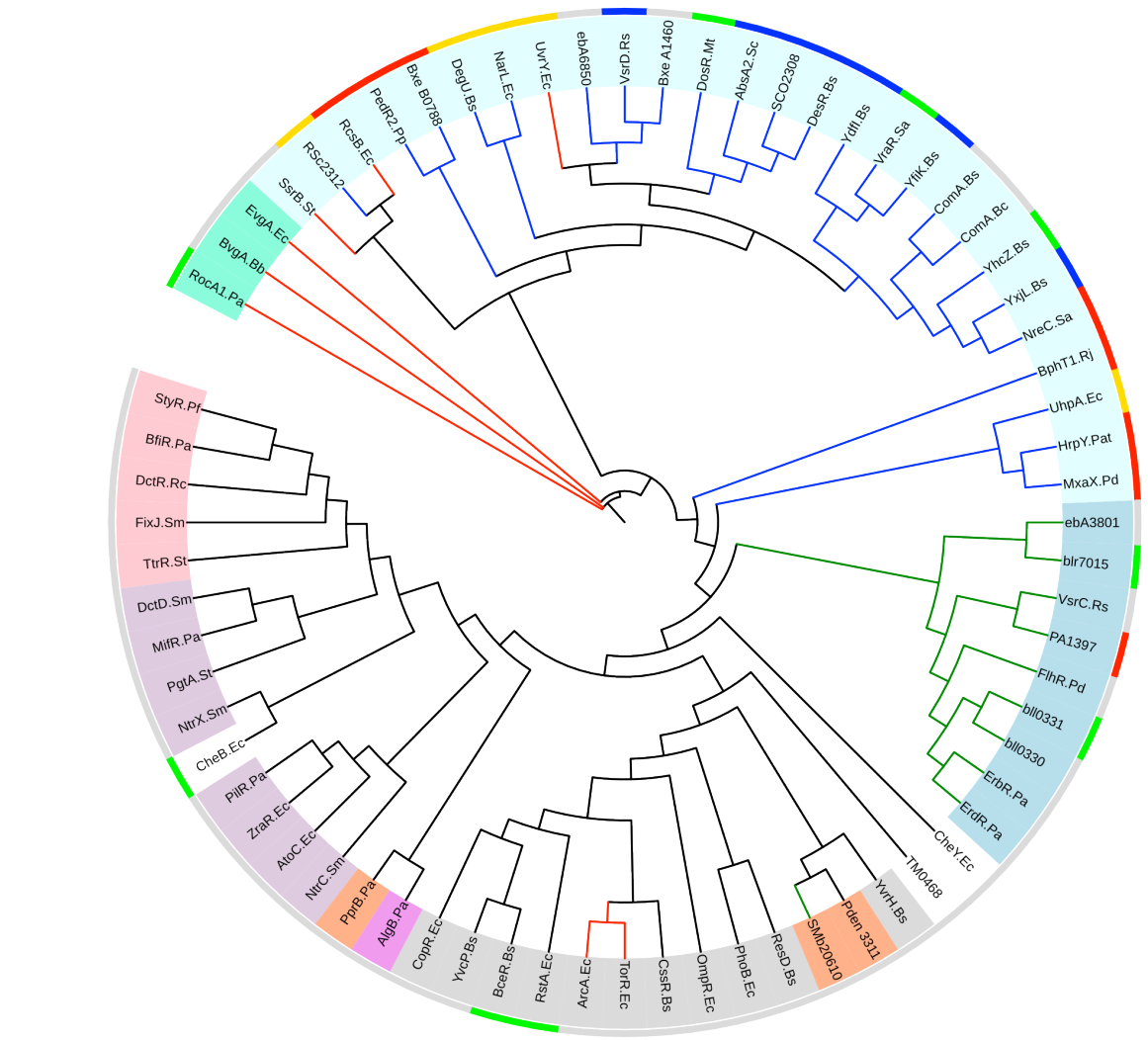


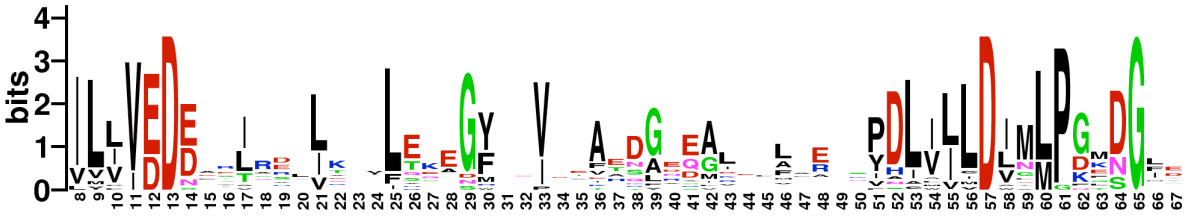
Fig. S1. Representative phylogram for receiver domains, The input file contained 86 sequences, and was constructed through the subtree pruning and regrafting method (see Materials and Methods for details). The resulting log likelihood value is –19,646.6. For presentation clarity, the tree was manually "pruned" in iTOL to remove closely-similar sequences, leaving the 70 sequences shown (see **Table S2**).

- FixJ-StyR family
- NtrC-DctD family
- OmpR-PhoB family
- NarL-VraR family
- BvgA subgroup
- FlhR subgroup
- outgroups
- Fis output domain
- GerE output domain

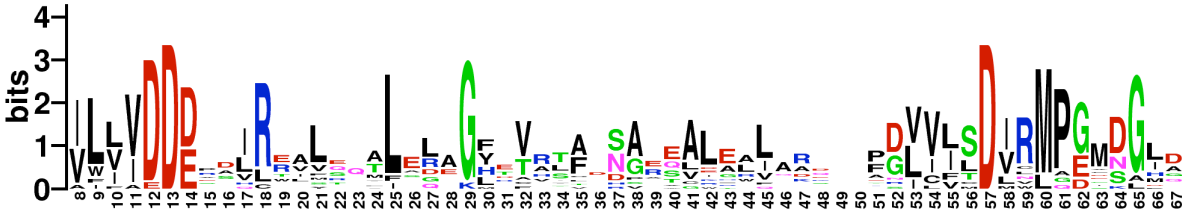
- T+1 = G or A
- T+1 = S
- T+1 = T
- T+1 = V or I
- T+1 = M or L

- HisKA sensor
- HisKA_3 sensor
- bipartite sensor
- tripartite sensor

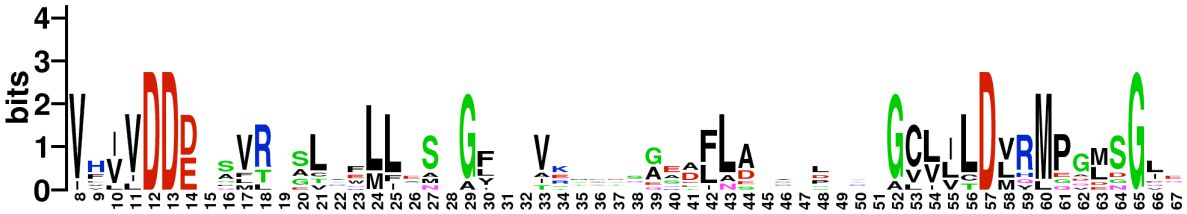
A. OmpR-PhoB Family (N-terminal half)



B. NtrC-DctD Family (N-terminal half)



C. FixJ-StyR Family (N-terminal half)



D. NarL-VraR Family (N-terminal half)

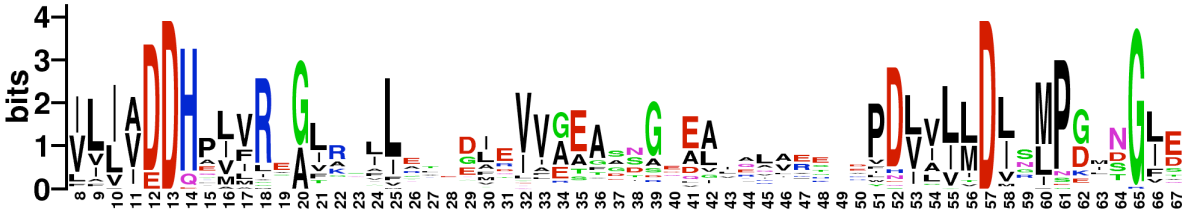
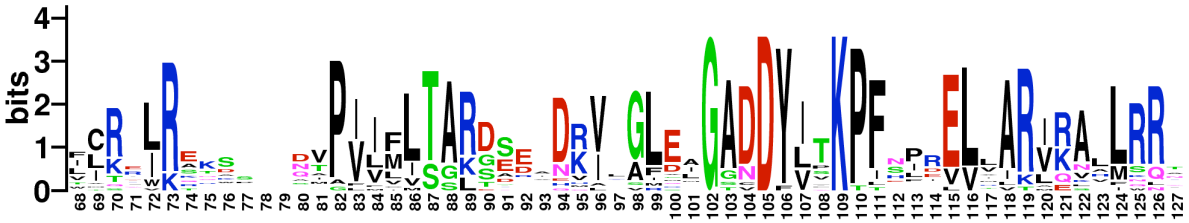
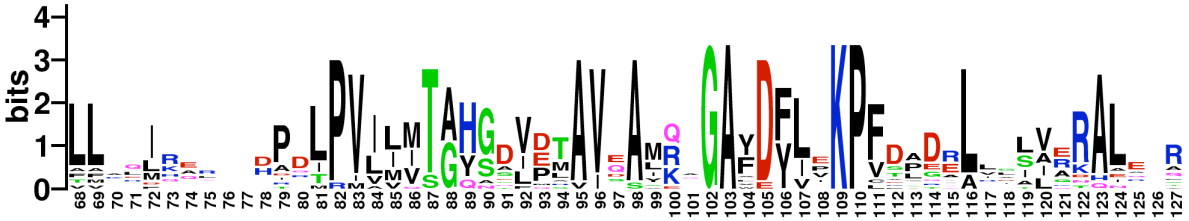


Fig. S2. Receiver domain sequence logos. Sequences are those depicted in **Fig. S1**. Numbering corresponds to that for CheY.Ec. See Materials and Methods for details of logo construction.

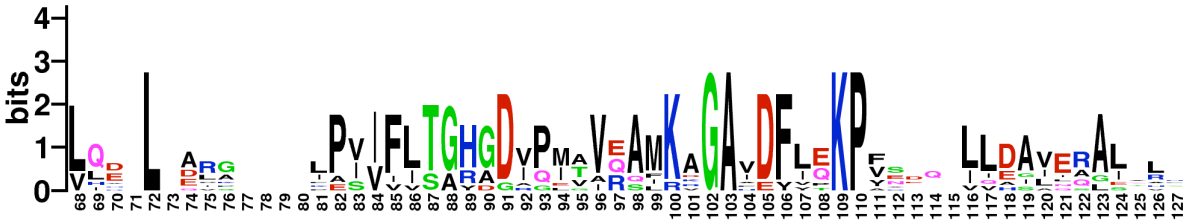
A. OmpR-PhoB Family (C-terminal half)



B. NtrC-DctD Family (C-terminal half)



C. FixJ-StyR Family (C-terminal half)



D. NarL-VraR Family (C-terminal half)

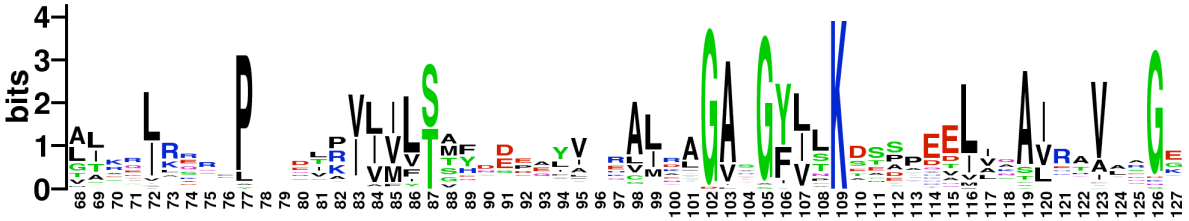


Fig. S2 (continued). Receiver domain sequence logos.

A	Class	Genus	Species	Strain	Sensor	Regulator	Alias Sensor	Alias Regul.	Locus Tag Sensor	Locus Tag Regulator	Accession Regul.	B C D	E F	G H I	J K L
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	CheA	CheY.Ec	—	—	b1888	b1882	NP_416396	D D F	**T** A	S G Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	CheA	CheB.Ec	—	—	b1888	b1883	NP_416397	D D S	S S	I D F	K P L
☑	Thermotogales	*Thermotoga*	*maritima*	MSB8	HK853	RR468	—	—	TM0853	TM0468	NP_228278	D D S	**T** A	R K V	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**BceS.Bs**	**BceR.Bs**	YtsB	YtsA	BSU30390	BSU30400	NP_390918	E D D	S S	D D F	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**CssS.Bs**	**CssR.Bs**	YvqB	YvqA	BSU33020	BSU33010	NP_391181	E D E	S A	N D Y	K P F
	Firmicutes	*Bacillus*	*subtilis*	168	**PhoR.Bs**	**PhoP.Bs**	—	—	BSU29100	BSU29110	NP_390789	D D E	T A	D D Y	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**ResE.Bs**	**ResD.Bs**	—	—	BSU23110	BSU23120	NP_390193	D D E	T A	D D Y	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**WalK.Bs**	**WalR.Bs**	YycG	YycF	BSU40400	BSU40410	NP_391921	D D E	T A	D D Y	K P F
	Firmicutes	*Bacillus*	*subtilis*	168	YbdK.Bs	**YbdJ.Bs**	—	—	BSU02010	BSU02000	NP_388082	E D D	S A	E D Y	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YcbM.Bs**	**YcbL.Bs**	—	—	BSU02560	BSU02550	NP_388137	E D D	S A	D D Y	K P F
	Firmicutes	*Bacillus*	*subtilis*	168	**YclK.Bs**	**YclJ.Bs**	—	—	BSU03760	BSU03750	NP_388257	E D N	T A	D D Y	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YkoH**	**YkoG**	—	—	BSU13260	BSU13250	NP_389208	E D E	T A	N D Y	K P F
	Firmicutes	*Bacillus*	*subtilis*	168	**YrkQ.Bs**	**YrkP.Bs**	—	—	BSU26420	BSU26430	NP_390520	E D D	T A	D D Y	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YvcQ.Bs**	**YvcP.Bs**	—	—	BSU34710	BSU34720	NP_391352	E D D	S A	D D Y	K P F
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YvrG.Bs**	**YvrH.Bs**	—	—	BSU33210	BSU33221	NP_391202	D D E	T A	D D Y	K P F
	Firmicutes	*Bacillus*	*subtilis*	168	**YxdK.Bs**	**YxdJ.Bs**	—	—	BSU39650	BSU39660	NP_391845	E D S	S A	D D Y	K P F
☑	Alphaproteobact.	*Paracoccus*	*denitrificans*	Pd1222	Pden_3312	**Pden_3311**	—	—	Pden_3312	Pden_3311	ABL71387	E D E	T A	D D Y	K P I
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMb20609 [REC]**	**SMb20610**	—	—	SMb20609	SMb20610	NP_438062	D D S	T G	V D Y	K P I
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**ArcB.Ec [Hpt]**	**ArcA.Ec**	—	—	b3210	b4401	NP_418818	E D E	T G	D D Y	K P F
	Gammaproteobact.	*Escherichia*	*coli*	K-12	**BaeS.Ec**	**BaeR.Ec**	—	—	b2078	b2079	NP_416583	E D E	T A	D D Y	K P Y
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**CopS.Ec**	**CopR.Ec**	YedV	YedW	b1968	b1969	NP_416478	E D N	T A	N D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**CpxA.Ec**	**CpxR.Ec**	—	—	b3911	b3912	NP_418348	D D D	T A	D D Y	K P F
	Gammaproteobact.	*Escherichia*	*coli*	K-12	**CreC.Ec**	**CreB.Ec**	—	—	b4399	b4398	NP_418815	E D E	T A	D D Y	K P F
	Gammaproteobact.	*Escherichia*	*coli*	K-12	**CusS.Ec**	**CusR.Ec**	YbcZ	YlcA	b0570	b0571	NP_415103	E D E	T A	D D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**KdpD.Ec**	**KdpE.Ec**	—	—	b0695	b0694	NP_415222	E D E	S A	D D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**EnvZ.Ec**	**OmpR.Ec**	—	—	b3404	b3405	NP_417864	D D D	T A	D D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**PhoR.Ec**	**PhoB.Ec**	—	—	b0400	b0399	NP_414933	E D E	T A	D D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**PhoQ.Ec**	**PhoP.Ec**	—	—	b1129	b1130	NP_415648	E D N	T A	D D Y	K P F
	Gammaproteobact.	*Escherichia*	*coli*	K-12	**PmrB.Ec**	**PmrA.Ec**	BasS	BasR	b4112	b4113	NP_418537	E D D	T A	D D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**QseC.Ec**	**QseB.Ec**	YgiY	YgiX	b3026	b3025	NP_417497	E D D	T A	D D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**RstB.Ec**	**RstA.Ec**	—	—	b1609	b1608	NP_416125	E D D	T S	C D Y	K T T
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**TorS.Ec [Hpt]**	**TorR.Ec**	—	—	b0993	b0995	NP_415515	E D E	T G	D D Y	K P L
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**KinB.Pa**	**AlgB.Pa**			PA5484	PA5483	NP_254170	D D E	T A	V D Y	K P C
	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**FixL.Bj**	**FixJ.Bj**	—	—	bll2760	bll2759	NP_769399	D D D	T G	V D F	K P F
	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**NodV,Bj**	**NodW.Bj**	—	—	bll1715	bll1714	NP_768354	E D D	T G	V D F	K P F
	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**NwsA.Bj**	**NwsB.Bj**	—	—	blr4773	blr4774	NP_771414	D D D	T G	V D F	K P V
☑	Alphaproteobact.	*Rhodobacter*	*capsulatus*	—	**DctS.Rc**	**DctR.Rc**	—	—	—	—	P37740	D D E	T G	F D F	K P F
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**FixL.Sm**	**FixJ.Sm**	—	—	SMa1229	SMa1227	NP_435915	D D E	T G	V D F	K P F
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMa0939**	**SMa0940**	—	—	SMa0939	SMa0940	NP_435754	D D D	T G	H D F	K P A
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMb20868**	**SMb20869**	—	—	SMb20868	SMb20869	NP_437703	D D D	T G	V D F	K P V
	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**DctS.Aa**	**DctR.Aa**	—	—	ebA4156	ebA4154	CAI08476	D D D	T G	F D F	K P F
	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**ebA126**	**ebA125**	—	—	ebA126	ebA125	CAI06190	D D D	T G	V D F	K P F
	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**Tcs2.Aa**	**Tcr2.Aa**	—	—	c1A81	c1A82	CAI07439	D D E	S A	M D F	K P F
☑	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**Tcs3.Aa**	**Tcr3.Aa**	TdiS	TdiR	c2A301	c2A300	CAI07155	D D E	S A	I D F	K P Y
	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**TtrS.Aa**	**TtrR.Aa**	—	—	ebA3063	ebA3060	CAI07852	D D D	T G	S D F	K P F
	Betaproteobact.	*Burkholderia*	*xenovorans*	LB400	**Bxe_A1540**	**Bxe_A1539**	—	—	Bxe_A1540	Bxe_A1539	ABE31416	D D D	T G	M D F	K P F
	Betaproteobact.	*Ralstonia*	*solanacearum*	GMI1000	**RSc1598**	**RSc1597**	—	—	RSc1598	RSc1597	CAD15299	D D D	T G	V D F	K P F
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**BfiS.Pa**	**BfiR.Pa**	—	—	PA4197	PA4196	NP_252885	D D D	S G	V D F	K P Y
	Gammaproteobact.	*Pseudomonas*	*putida*	KT2440	**PP_3552**	**PP_3551**	—	—	PP_3552	PP_3551	NP_745688	D D D	S G	C D F	K P Y
	Gammaproteobact.	*Pseudomonas*	*putida*	KT2440	**PP_5242**	**PP_5241**	—	—	PP_5242	PP_5241	NP_747342	D D D	T G	V E F	K P F
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**PprA.Pa**	**PprB.Pa**	—	—	PA4293	PA4296	NP_252986	D D E	T G	A D Y	K P V
☑	Gammaproteobact.	*Pseudomonas*	*fluorescens*	ST	**StyS.Pf**	**StyR.Pf**	—	—	—	—	1YIO_A	D D D	T A	I E F	K P F
☑	Gammaproteobact.	*Salmonella*	*typhimurium*	LT2	**TtrS.St**	**TtrR.St**	—	—	STM1386	STM1387	NP_460352	D D D	T G	V D F	K P V
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**DctB.Sm**	**DctD.Sm**	—	—	SMb20612	SMb20613	NP_438065	D D D	T G	Y D F	K P F
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**NtrB.Sm**	**NtrC.Sm**	—	—	SMc01042	SMc01043	NP_385565	D D D	S A	Y D Y	K P F
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**NtrY.Sm**	**NtrX.Sm**	—	—	SMc01044	SMc01045	NP_385567	D D E	S G	Y D F	K P F
☑	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMb21201**	**SMb21200**	—	—	SMb21201	SMb21200	NP_437405	D D E	T G	Y D F	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**AtoS.Ec**	**AtoC.Ec**	—	—	b2219	b2220	NP_416724	D D E	T A	F D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**NtrB.Ec**	**NtrC.Ec**	—	—	b3869	b3868	NP_418304	D D D	T A	F D Y	K P F
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**ZraS.Ec**	**ZraR.Ec**	—	—	b4003	b4004	NP_418432	D D D	T A	L D Y	K P L
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**FleS.Pa**	**FleR.Pa**	—	—	PA1098	PA1099	NP_249790	E D D	T A	A D Y	K P F
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**MifS.Pa**	**MifR.Pa**	—	—	PA5512	PA5511	NP_254198	D D E	T G	Y D F	K P F
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**NtrB.Pa**	**NtrC.Pa**	—	—	PA5124	PA5125	NP_253812	D D D	T A	F E Y	K P F
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**PA5165**	**PA5166**	—	—	PA5165	PA5166	NP_253853	D D D	T G	Y D F	K P F
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**PilS.Pa**	**PilR.Pa**	—	—	PA4546	PA4547	NP_253237	D D E	T A	F D F	K P V
☑	Gammaproteobact.	*Salmonella*	*typhimurium*	LT2	**PgtB.St**	**PgtA.St**	—	—	STM2397	STM2396	NP_461337	D D D	T G	W D F	K P V
☑	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**blr0329 [REC]**	**bll0330**	—	—	blr0329	bll0330	NP_766970	E D H	S G	S G Y	K S S
☑	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**blr0329 [REC]**	**bll0331**	—	—	blr0329	bll0331	NP_766971	D D H	S S	A G Y	K S T
	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**bll1199 [REC]**	**blr1194**	—	—	bll1199	blr1194	NP_767834	D D H	S A	S G F	K R F
	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**bll5889 [REC]**	**bll5888**	—	—	bll5889	bll5888	NP_772528	D D H	S A	S G Y	K S L
☑	Alphaproteobact.	*Bradyrhizobium*	*japonicum*	USDA 110	**bll7016 [REC]**	**blr7015**	—	—	bll7016	blr7015	NP_773655	D D H	S S	L G F	K T T
☑	Alphaproteobact.	*Paracoccus*	*denitrificans*	Pd1222	**FlhS.Pd [REC]**	**FlhR.Pd**	—	—	Pden_2352	Pden_2354	ABL70444	D D H	S A	Q G Y	K S L
	Alphaproteobact.	*Paracoccus*	*denitrificans*	Pd1222	**Pden_4348 [REC]**	**Pden_4349**	—	—	Pden_4348	Pden_4349	ABL72413	D D H	S M	D G F	K A V
	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMb20164 [REC]**	**SMb20162**	—	—	SMb20164	SMb20162	NP_436702	D D H	S A	A G F	K D I
	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMc02249 [REC]**	**SMc02248**	—	—	SMc02249	SMc02248	NP_384658	D D H	S A	A G F	K S A
☑	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**ebA3794 [REC]**	**ebA3801**	—	—	ebA3794	ebA3801	CAI08265	E D H	S A	S G F	K A S
☑	Betaproteobact.	*Ralstonia*	*solanacearum*	GMI1000	**VsrB.Rs [REC]**	**VsrC.Rs**	—	—	RSc2456	RSc2455	CAD16162	D D H	S A	I G F	K H A
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**ErcS.Pa [REC]**	**ErbR.Pa**	—	AgmR	PA1992	PA1978	NP_250668	D D H	S A	V G F	K S S
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**ErcS'.Pa [REC]**	**ErdR.Pa**	—	—	PA1976	PA3604	NP_252294	D D H	S A	S G F	K S S
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**PA1396 [REC]**	**PA1397**	—	—	PA1396	PA1397	NP_250088	D D H	S M	D G F	K S I
☑	Betaproteobact.	*Bordetella*	*bronchiseptica*	RB50	**BvgS.Bb [Hpt]**	**BvgA.Bb**	—	—	BB2995	BB2994	CAE33486	D D H	T G	A G F	K H E
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**EvgS.Ec [Hpt]**	**EvgA.Ec**	—	—	b2370	b2369	NP_416870	D D H	S A	N G F	K K E
☑	Gammaproteobact.	*Pseudomonas*	*aeruginosa*	PAO1	**RocS1.Pa [Hpt]**	**RocA1.Pa**	—	—	PA3946	PA3948	NP_252637	D D H	T S	R G F	K E E
	Gammaproteobact.	*Pseudomonas*	*putida*	KT2440	**PP_3413 [Hpt]**	**PP_3412**	—	—	PP_3413	PP_3412	NP_745550	D D H	T G	G A F	K E E

A	Class	Genus	Species	Strain	Sensor	Regulator	Alias Sensor	Alias Regul.	Locus Tag Sensor	Locus Tag Regulator	Accession Regul.	B	C	D	E	F	G	H	I	J	K	L
☑	Actinobacteria	*Mycobacterium*	*tuberculosis*	H37Rv	**DosS.Mt**	**DosR.Mt**	—	—	Rv3132c	Rv3133c	NP_217649	D	D	H	T	S	S	G	Y	K	D	I
	Actinobacteria	*Mycobacterium*	*smegmatis*	MC2 155	**MSMEG_6238**	**MSMEG_6236**	—	—	MSMEG_6238	MSMEG_6236	YP_890456	D	D	H	T	T	R	G	Y	K	D	V
☑	Actinobacteria	*Rhodococcus*	*jostii*	RHA1	**BphS1.Rj**	**BphT1.Rj**	—	—	RHA1_ro08052	RHA1_ro08051	ABG99098	D	D	H	S	M	R	G	Y	K	E	C
☑	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**AbsA1.Sc**	**AbsA2.Sc**	—	—	SCO3225	SCO3226	NP_627440	D	D	E	T	T	A	G	F	K	A	S
	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**SCO1369**	**SCO1370**	—	—	SCO1369	SCO1370	NP_625654	D	D	H	T	T	T	G	Y	K	D	A
☑	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**SCO2307**	**SCO2308**	—	—	SCO2307	SCO2308	NP_626556	E	D	Q	T	T	A	G	F	K	D	G
	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**SCO4073**	**SCO4072**	—	—	SCO4073	SCO4072	NP_628253	D	D	E	T	S	R	G	F	K	T	V
	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**SCO5824**	**SCO5825**	—	—	SCO5824	SCO5825	NP_629948	D	D	Q	T	T	R	G	Y	K	D	A
	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**SCO6139**	**SCO6140**	—	—	SCO6139	SCO6140	NP_630245	D	D	Q	T	T	S	G	F	K	D	A
	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**SCO6163**	**SCO6162**	—	—	SCO6163	SCO6162	NP_630267	D	D	H	T	M	C	G	Y	K	S	V
	Actinobacteria	*Streptomyces*	*coelicolor*	A3(2)	**SCO6668**	**SCO6667**	—	—	SCO6668	SCO6667	NP_630742	D	D	Q	T	T	S	G	F	K	D	V
	Firmicutes	*Bacillus*	*anthracis*	Ames Ancest.	**GBAA1792**	**GBAA1791**	—	—	GBAA1792	GBAA1791	AAT30904	D	D	N	T	T	K	G	Y	K	N	N
☑	Firmicutes	*Bacillus*	*cereus*	ATCC 14579	**ComP.Bc**	**ComA.Bc**	—	—	BC0881	BC0882	NP_830668	D	D	H	T	G	L	G	V	K	T	S
☑	Firmicutes	*Bacillus*	*subtilis*	168	**ComP.Bs**	**ComA.Bs**	—	—	BSU31690	BSU31680	NP_391046	D	D	H	T	G	H	G	A	K	T	E
☑	Firmicutes	*Bacillus*	*subtilis*	168	**DegS.Bs**	**DegU.Bs**	—	—	BSU35500	BSU35490	NP_391429	D	D	H	S	I	R	G	Y	K	E	M
☑	Firmicutes	*Bacillus*	*subtilis*	168	**DesK.Bs**	**DesR.Bs**	YocF	YocG	BSU19190	BSU19200	NP_389801	E	D	Q	T	T	K	G	Y	K	D	S
	Firmicutes	*Bacillus*	*subtilis*	168	**LiaS.Bs**	**LiaR.Bs**	YvqE	YvqC	BSU33090	BSU33080	NP_391188	D	D	H	T	S	L	S	Y	K	T	S
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YdfH.Bs**	**YdfI.Bs**	—	—	BSU05410	BSU05420	NP_388423	D	D	H	T	T	K	G	Y	K	D	T
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YfiJ.Bs**	**YfiK.Bs**	—	—	BSU08290	BSU08300	NP_388711	D	D	Q	T	T	S	G	Y	K	D	M
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YhcY.Bs**	**YhcZ.Bs**	—	—	BSU09320	BSU09330	NP_388814	D	D	H	T	S	K	A	Y	K	D	T
☑	Firmicutes	*Bacillus*	*subtilis*	168	**YxjM.Bs**	**YxjL.Bs**	—	—	BSU38900	BSU38910	NP_391770	D	D	Q	T	T	V	G	Y	K	D	T
	Firmicutes	*Clostridium*	*acetobutylicum*	ATCC 824	**CA_C0173**	**CA_C0174**	—	—	CA_C0173	CA_C0174	NP_346816	D	D	Q	T	T	D	G	Y	K	D	A
	Firmicutes	*Clostridium*	*acetobutylicum*	ATCC 824	**CA_C1454**	**CA_C1455**	—	—	CA_C1454	CA_C1455	NP_348083	D	D	Q	T	T	S	G	Y	K	G	V
	Firmicutes	*Clostridium*	*acetobutylicum*	ATCC 824	**CA_C2253**	**CA_C2254**	—	—	CA_C2253	CA_C2254	NP_348871	D	D	Q	T	T	S	G	Y	K	D	S
	Firmicutes	*Clostridium*	*acetobutylicum*	ATCC 824	**CA_C2940**	**CA_C2939**	—	—	CA_C2940	CA_C2939	NP_349541	D	D	E	T	T	N	G	Y	K	D	T
	Firmicutes	*Clostridium*	*acetobutylicum*	ATCC 824	**CA_C3430**	**CA_C3429**	—	—	CA_C3430	CA_C3429	NP_350019	D	D	H	T	T	K	G	Y	K	D	T
☑	Firmicutes	*Staphylococcus*	*aureus*	Mu50	**NreB.Sa**	**NreC.Sa**	—	—	SAV2392	SAV2391	BAB58553	D	D	H	T	M	K	G	Y	K	N	A
	Firmicutes	*Staphylococcus*	*aureus*	Mu50	**SAV1321**	**SAV1322**	—	—	SAV1321	SAV1322	BAB57484	E	D	Q	T	T	D	A	Y	K	E	R
	Firmicutes	*Staphylococcus*	*aureus*	Mu50	**SAV1849**	**SAV1848**	—	—	SAV1849	SAV1848	BAB58010	D	D	H	T	S	D	G	Y	K	D	V
☑	Firmicutes	*Staphylococcus*	*aureus*	Mu50	**VraS.Sa**	**VraR.Sa**	—	—	SAV1885	SAV1884	BAB58046	D	D	H	T	S	D	S	Y	K	T	T
☑	Alphaproteobact.	*Paracoccus*	*denitrificans*	Pd1222	**MxaY.Pd**	**MxaX.Pd**	—	—	Pden_2991	Pden_2990	ABL71073	D	D	H	T	M	N	G	I	K	D	S
	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMb20077**	**SMb20078**	—	—	SMb20077	SMb20078	NP_436618	D	D	H	T	A	S	G	L	K	E	Y
	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMb20467**	**SMb20468**	—	—	SMb20467	SMb20468	NP_436990	D	D	H	T	A	S	G	I	K	E	Q
	Alphaproteobact.	*Sinorhizobium*	*meliloti*	1021	**SMb21519**	**SMb21520**	—	—	SMb21519	SMb21520	NP_437890	D	D	H	T	V	K	G	Y	K	G	I
	Betaproteobact.	*Acidovorax*	*ebreus*	TPSY	**Dtpsy_2833**	**Dtpsy_2832**	—	—	Dtpsy_2833	Dtpsy_2832	ACM34266	D	D	H	S	G	S	G	Y	K	E	C
	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**ebA3729**	**ebA3727**	—	—	ebA3729	ebA3727	CAI08225	D	D	H	T	G	R	G	V	K	E	E
	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**ebA5715**	**ebA5719**	—	—	ebA5715	ebA5719	CAI09371	D	D	H	S	M	N	A	Y	K	D	V
☑	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**ebA6844**	**ebA6850**	—	—	ebA6844	ebA6850	CAI10037	D	D	H	S	A	T	G	Y	K	A	E
	Betaproteobact.	*Aromatoleum*	*aromaticum*	EbN1	**Tcs1.Aa**	**Tcr1.Aa**	—	—	c1A75	c1A77	CAI07437	D	D	H	T	M	A	G	Y	K	G	K
	Betaproteobact.	*Bordetella*	*bronchiseptica*	RB50	**BB1122**	**BB1120**	—	—	BB1122	BB1120	CAE31618	D	D	H	S	M	S	G	Y	K	D	M
☑	Betaproteobact.	*Burkholderia*	*xenovorans*	LB400	**Bxe_A1456**	**Bxe_A1460**	—	—	Bxe_A1456	Bxe_A1460	ABE31494	D	D	H	S	G	N	G	Y	K	D	C
	Betaproteobact.	*Burkholderia*	*xenovorans*	LB400	**Bxe_A1814**	**Bxe_A1815**	—	—	Bxe_A1814	Bxe_A1815	ABE31147	D	D	H	T	S	H	S	F	K	S	M
	Betaproteobact.	*Burkholderia*	*xenovorans*	LB400	**Bxe_A1851**	**Bxe_A1850**	—	—	Bxe_A1851	Bxe_A1850	ABE31115	D	D	H	T	M	S	G	Y	K	E	S
☑	Betaproteobact.	*Burkholderia*	*xenovorans*	LB400	**Bxe_B0789**	**Bxe_B0788**	—	—	Bxe_B0789	Bxe_B0788	ABE35166	D	D	H	S	M	S	G	Y	K	D	S
	Betaproteobact.	*Burkholderia*	*xenovorans*	LB400	**Bxe_B2472**	**Bxe_B2471**	—	—	Bxe_B2472	Bxe_B2471	ABE33520	D	D	H	S	V	L	G	Y	K	A	S
	Betaproteobact.	*Ralstonia*	*solanacearum*	GMI1000	**RSc0191**	**RSc0192**	—	—	RSc0191	RSc0192	CAD13720	D	D	H	T	M	A	A	Y	K	E	S
☑	Betaproteobact.	*Ralstonia*	*solanacearum*	GMI1000	**RSc2311**	**RSc2312**	—	—	RSc2311	RSc2312	CAD16019	E	D	H	T	V	D	G	Y	K	D	A
☑	Betaproteobact.	*Ralstonia*	*solanacearum*	GMI1000	**VsrA.Rs**	**VsrD.Rs**	—	—	RSc0289	RSc0292	CAD13820	D	D	H	S	T	S	G	Y	K	E	S
	Betaproteobact.	*Rhodoferax*	*ferrireducens*	T118	**Rfer_0781**	**Rfer_0782**	—	—	Rfer_0781	Rfer_0782	ABD68532	D	D	H	T	A	R	G	V	K	S	E
	Deltaproteobact.	*Anaeromyxobacter*	*sp.*	Fw109-5	**Anae109_3354**	**Anae109_3355**	—	—	Anae109_3354	Anae109_3355	ABS27539	D	D	H	T	A	R	G	L	K	D	A
	Gammaproteobact.	*Azotobacter*	*vinelandii*	DJ	**Avin_17340**	**Avin_17330**	—	—	Avin_17340	Avin_17330	ACO77946	D	D	H	S	M	S	G	Y	K	D	V
	Gammaproteobact.	*Azotobacter*	*vinelandii*	DJ	**Avin_41030**	**Avin_41040**	—	—	Avin_41030	Avin_41040	ACO80238	D	D	H	S	M	R	G	Y	K	D	A
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**NarX.Ec**	**NarL.Ec**	—	—	b1222	b1221	NP_415739	D	D	H	S	V	D	G	Y	K	D	M
	Gammaproteobact.	*Escherichia*	*coli*	K-12	**NarQ.Ec**	**NarP.Ec**	—	—	b2469	b2193	NP_416697	D	D	H	T	V	D	G	Y	K	D	S
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**RcsCD.Ec [Hpt]**	**RcsB.Ec**	—	—	b2216	b2217	NP_416721	D	D	H	T	M	E	G	I	K	Q	G
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**UhpB.Ec**	**UhpA.Ec**	—	—	b3668	b3669	NP_418125	D	D	H	S	V	R	G	F	K	R	C
☑	Gammaproteobact.	*Escherichia*	*coli*	K-12	**BarA [Hpt]**	**UvrY.Ec**	GacS	GacA	b2786	b1914	NP_416424	D	D	H	T	V	A	G	Y	K	G	A
☑	Gammaproteobact.	*Pectobacterium*	*atrosepticum*	SCRI1043	**HrpX.Pat**	**HrpY.Pat**	—	—	ECA2088	ECA2089	CAG74991	D	D	H	S	M	R	G	Y	K	D	K
☑	Gammaproteobact.	*Pseudomonas*	*putida*	KT2440	**PedS2.Pp**	**PedR2.Pp**	—	—	PP_2671	PP_2672	NP_744816	D	D	H	S	M	S	G	Y	K	N	S
	Gammaproteobact.	*Pseudomonas*	*putida*	KT2440	**PP_0409**	**PP_0410**	—	—	PP_0409	PP_0410	NP_742576	E	D	H	S	M	A	G	Y	K	D	S
	Gammaproteobact.	*Pseudomonas*	*putida*	KT2440	**PP_2127**	**PP_2126**	—	—	PP_2127	PP_2126	NP_744275	D	D	H	S	M	H	G	Y	K	D	T
☑	Gammaproteobact.	*Salmonella*	*typhimurium*	LT2	**SsrA [Hpt]**	**SsrB**	—	—	STM1392	STM1391	NP_460356	D	D	H	T	A	N	G	Y	K	S	S
	Gammaproteobact.	*Xanthomonas*	*campestris*	ATCC 33913	**XCC1127**	**XCC1128**	—	—	XCC1127	XCC1128	NP_636503	D	D	Q	T	T	K	G	Y	K	D	V
	Gammaproteobact.	*Xanthomonas*	*campestris*	ATCC 33913	**XCC2179**	**XCC2180**	—	—	XCC2179	XCC2180	NP_637536	D	D	H	T	T	C	G	Y	K	S	A
	Gammaproteobact.	*Xanthomonas*	*campestris*	ATCC 33913	**XCC3910**	**XCC3909**	—	—	XCC3910	XCC3909	NP_639249	E	D	Q	T	T	A	G	Y	K	D	A

Columns

A Included in the phylogram constructed for Fig. S1.
B Residue at position corresponding to CheY-12.
C Residue at position corresponding to CheY-13.
D Residue at position corresponding to CheY-14 (DD+1).
E Residue at position corresponding to CheY-87.
F Residue at position corresponding to CheY-88 (T+1).
G Residue at position corresponding to CheY-104.
H Residue at position corresponding to CheY-105.
I Residue at position corresponding to CheY-106.
J Residue at position corresponding to CheY-109.
K Residue at position corresponding to CheY-110 (K+1).
L Residue at position corresponding to CheY-111

HPK 1	**OmpR-PhoB**
HPK 2	**FixJ-StyR**
HPK 3	**NtrC-DctD**
HPK 4	**NarL-VraR**
HPK 7	**GerE domain**
	Fis domain