CRISPR/Cas9-induced knockout and knock-in mutations in Chlamydomonas reinhardtii

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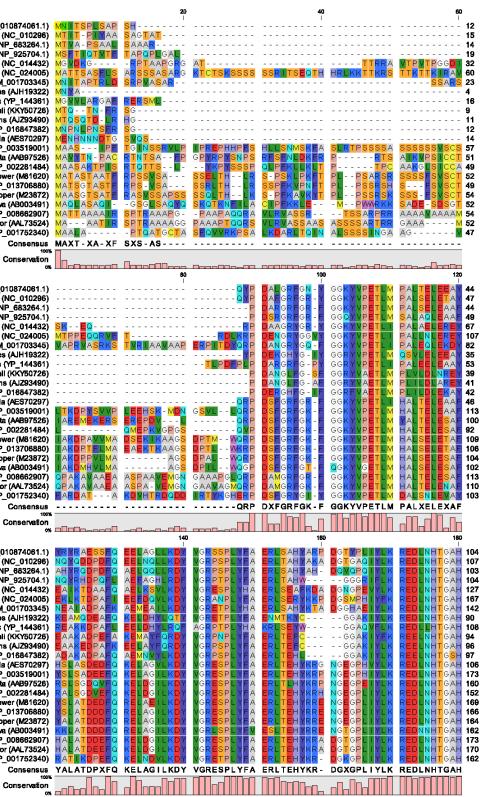
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Synechocystis sp. (WP_010874061.1) Mcrocystis eeruginosa (NC_010296) Thermosynechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) Chlamydomonas reinhardtil (XM_001703345) Bacilius mycoides (AJH19322) Thermus thermophilus (YP_144361) Pseudomonas amygdali (KKY50726) Pseudomonas fluorescens (AJZ93490) Bradyrhizobium elkanii (WP_016847382) Medicago truncatula (AES70297) Glycine max (XP_003519001) Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484) Arabidopsis thaliana_lower (M81620) Brassica napus (XP_013706880) Arabidopsis thaliana_upper (M23872) Oryza sativa (AB003491) Zea mays (XP 008662907) Sorghum bicolor (AAL73524) Physcomitrella patens (XP_001752340) Conservation

Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010296) Thermosynechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) Chlamydomonas reinhardtii (XM_001703345) Badilus mycoides (AIH19322) Themus thermophilus (YP_144361) Pseudomonas amygdall (KKY50726) Pseudonionas invigoan (Art50720) Pseudomonas fluorescens (Al293490) Bradyrhizobium elkani (WP_018847382) Medicago truncatula (AES70297) Glycine max (XP_003519001) Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484) Arabidopsis thellena_lower (M81620) Brassica napus (XP_013706880) Arabidopsis thaliana_upper (M23872) Oryza sativa (AB003491) Zea mays (XP_008662907) Sorghum bicolor (AAL73524) Physcomitrella patens (XP_001752340) FARD Consensus

Synechocystis sp. (WP_010874061.1) Mcrocystis aeruginosa (NC_010296) Thermosynechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1) Gioeobadder Wolaceus (NP_925/04.1) Ostrecococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) Chiamydomonas reinhardili (XM_001703345) Bacilius mycoldes (AJH19322) Themus thermophilus (YP_144361) Pseudomonas amygdali (KKY50726) Pseudomonas fluorescens (AJ293490) Bradyrhizobium elkanii (WP_016847382) Medicago truncatula (AES70297) Glycine max (XP_003519001) Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484) Arabidopsis thaliana_lower (M81620) Brassica napus (XP_013706880) Arabidopsis thallana_upper (M23872) Oryza sativa (AB003491) Zea mays (XP_008662907) Sorphum bicolor (AAL73524) Physcomitrella patens (XP_001752340) Consensus

		200		220		240
Synechocystis sp. (WP_010874061.1)	KINNAL GOVL		AETGAGQHG		GLECIIYMGV	
Mcrocystis aeruginosa (NC_010296) nechococcus elongatus (NP_683264.1)	KINNALGQVL KINNALGQVL	LAKRMGKKRI LAKRMG <mark>KQRI</mark>	IAETGAGQHG IAETGAGQHG	VATATVCARE Va <mark>tatvcar</mark> e	GLECVIYMGI GLQCVIYMGV	HDMERQELNV 167 QDMERQRLNV 163
Gioeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432)	K INN AL GQAL K INN AVGQAL	LAL <mark>RM</mark> GKRRI LAKRMGKKRI	IAETGAG <mark>Q</mark> HG IAETGAGQHG	VATATVCARF VATATVCARF	GLECIIYMGV GLECIIYMGA	HDIERQKLNV 164 Admerqklnv 187
Bathycoccus prasinos (NC_024005)	K INN AUGQAL	L A <mark>KRM</mark> GKKR I	I A <mark>ET</mark> GAG <mark>Q</mark> HG	VATAT VCARY	GLECIIYMGA	ADMERQKLNV 227
nydomonas reinhardtil (XM_001703345) Bacilius mycoides (AJH19322)	KINN <mark>SL</mark> GQAL KINNTIGQAL	L <mark>CKRLNKQRI</mark> LA <mark>VRM</mark> GKKKV	IAETGAGQHG VAETGAGQHG	VATATICA <mark>R</mark> L VatatvcaLF	G <mark>lkcivym</mark> ga G lecvifm g e	KDMERQALNV 202 EDVRRQKLNV 150
Thermus thermophilus (YP_144361)	KINNTLGQAL	LARR <mark>M</mark> G <mark>KRRV</mark>	IA <mark>ET</mark> GAG <mark>QH</mark> G	V <mark>sv</mark> a <mark>tv</mark> aalf	GL <mark>ECVVYM</mark> G <mark>E</mark>	EDVRRQALNV 168
Pseudomonas amygdali (KKY50726) Pseudomonas fluorescens (AJZ93490)	KINNCIGQVL KINNCIGQIL	LAKRMGKKRL LARRMGKKRI	IAETGAGMHG IAETGAGMHG	VATATVAARE VATATVAARE	GLPCVIYMGA GLQCVIYMGT	TDIERQQANV 154 TDIERQQANV 156
Bradyrhizobium elkanii (WP_016847382)	KVNNVLGQL <mark>M</mark>	VARR <mark>M</mark> GKKR I	I A <mark>ET</mark> GAG <mark>Q</mark> HG	VATATLCARF	GL <mark>ECVVYM</mark> GA	VDVERQQPNV 157
Medicago truncatula (AES70297) Givcine max (XP 003519001)	KINNAVAQAL KINNAVAQAL	LA <mark>KKLGKKR</mark> V LAKRLGKKRI	IAETGAGQHG IAETGAGQHG	VATATVCARF VATATVCARF	GLECVVYMGA GLECIIYMGA	LDMERQALNV 166 QDMERQALNV 233
Camptotheca acuminata (AAB97526)	K I NN AVAQAL	LA <mark>KR</mark> LG <mark>KKR</mark> I	IA <mark>ET</mark> GAG <mark>QH</mark> G	VATA <mark>TV</mark> CA <mark>R</mark> F	G <mark>lqcviym</mark> ga	QDMERQALNV 220
Vitis vinifera (XP_002281484) Arabidopsis thaliana lower (M81620)	K INN AVAQAL K INN AVAQAL	LA <mark>KRL</mark> GK <mark>T</mark> RI LAKRLGKKRI	IAETGAGQHG IAETGAGQHG	VATATVCARF VATATVCARF	GLOCIVYMGA GLOCIIYMGA	QDMERQALNV 212 QDMERQALNV 229
Brassica napus (XP_013706880)	<mark>k inn</mark> ava <mark>q</mark> al	LA <mark>KR</mark> LG <mark>KKR</mark> I	I A <mark>ET</mark> GAG <mark>Q</mark> HG	VA <mark>TATVCAR</mark> F	GL <mark>QC</mark> II <mark>YM</mark> GA	QDMERQALNV 226
Arabidopsis thaliana_upper (M23872) Oryza sativa (AB003491)	KINNAVAQAL KINNAVAQAL	LAKRLGKKRI Sakrlgkkri	I AET GAGOHG I AET GAGOHG	VATATVCA <mark>RF</mark> VATATVCASF	GLECIIYMGA GLECIIYMGA	QDMERQALNV 224 QDMERQALNV 222
Zea mays (XP_008662907)	K INN AVAQAL	LAKRLGKORI	IA <mark>ET</mark> GAG <mark>QH</mark> G	VATATVCARF	GL <mark>QCIIYM</mark> GA	QDMERQALNV 233
Sorghum bicolor (AAL73524) Physcomitrella patens (XP_001752340)	K INN AVAQAL K INN ATAQAL	LAKRLGKORI LAKRIGKKRI	IAETGAGQHG IAETGAGQHG	VATATVCARE VATATVCARE	GLQCIIYMGA GLECIVYMGA	QDMERQALNV 230 QDMERQALNV 222
Consensus	KINNAVGQAL	LAKRXGKKRI	IAETGAGQHG	VATATVCARF	GLECIIYMGA	QDMERQALNV
Conservation						
0%		260		260		300
Synechocystis sp. (WP_010874061.1)	FRMNLLGARV	QPVTAGTGTL	KDATSEATRD	WVTNVETTHY	ILG <mark>SV</mark> AGPHP	YPMMVRDFHR 224
Mcrocystis aeruginosa (NC_010296) mechococcus elongatus (NP_683264.1)	FRMRLLGATV LRMRLLGA E V	QPVAAGTGTL APVSAGTGTL	KDATSEAIRD KDATSEAIRD	WVTNVETTHY WVTNVETTHY	ILG <mark>SV</mark> AGPHP ILG <mark>SV</mark> AGPHP	YPMMVRDFHA 227 YPMLVREFHA 223
Gloeobacter violaceus (NP_925704.1)	YRMKLLGA <mark>E</mark> V	R P VAAGTGTL	K <mark>datse</mark> a <mark>ir</mark> d	WVTHVETTHY	IL G <mark>S</mark> AAGPHP	YPLMVREFQA 224
Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005)	FRMRLLGATV FRMRLLGATV	RPVWAGTATL TPVRTGTSTL	KDATSEAIRD KDATSQAIRD	WVTNVEDTHY WVTNVETTHY	IL <mark>GSV</mark> AGPHP ILG <mark>SV</mark> AGPHP	YPMMVRDFHA 247 YPMMVRDFHS 287
nydomonas reinhardiii (XM_001703345)	FR <mark>MR</mark> L <mark>C</mark> GA <mark>E</mark> V	<mark>r p vhs</mark> gtat l	K <mark>da<u>ts</u>eaird</mark>	WVTNVETTHY	IL <mark>G<mark>S</mark>AAGPHP</mark>	YPMMVREFQS 262
Bacillus mycoides (AJH19322) Thermus thermophilus (YP_144361)	FRMELLGAKV FRMKLLGAEV	ESVAAGSGTL RPVAAGSRTL	KDA <mark>VNE</mark> ALRY KDATNEAIRD	WVSHVHDTHY WITNVRTTFY	I <mark>MGSVL</mark> GPHP	FPQIVRDFQS 210 YPMMVRDFQS 228
Pseudomonas amygdall (KKY50726)	FR <mark>MKLL</mark> GA <mark>E</mark> I	VPVTSGTGTL	K DA <mark>mn</mark> ealrd	WVTNVDDTFY	L I G <mark>T V</mark> AGPHP	YPAMVRDFQA 214
Pseudomonas fluorescens (AJZ93490) Bradyrhizobium elkanii (WP_016847382)	FRMKLLGAEV	IPMVAGTGTL VPMQSGARTL	KDAMNEALRD KDAMNDALRD	WVTNVDSTFY WVTNVHDTFY	LIGTVAGPHP CIGTVAGPHP	YPAMVRDFQA 216 YPMMVRDFQS 217
Medicago truncatula (AES70297)	FRMRLLGAEV	RAVHAGTATL	KDA <mark>tse</mark> a ird	WVTNVETTHY	TLG <mark>SVC</mark> GPHP	YPMMVREFHA 226
Glycine max (XP_003519001) Camptotheca acuminata (AAB97526)	FRMRLLGAEV	R P VH SGT AT L R AVH SGT AT L	KDATSEAIRD KDATSEAIRD	WVTNVETTHY WVTNVESTHY	ILG <mark>SV</mark> AGPHP ILG <mark>SV</mark> AGPHP	YPMMVREFHA 293 YPMMVREFHA 280
Vitis vinifera (XP_002281484)	FRMRLLGAEV	RAVHAGTATL	K <mark>d</mark> a <mark>tse</mark> a <mark>ir</mark> d	WVTNVETTHY	IL <mark>G<mark>S</mark>VAG<mark>PHP</mark></mark>	YPMMVRDFHA 272
Arabidopsis thallana_lower (M81620) Brassica napus (XP_013706880)	FRMRLLGAEV Frmrllgaev	RG <mark>VHS</mark> GTATL RGVHSGTATL	KDATSEALRD KDATSEALRD	WVTNVETTHY WVTNVETTHY	ILG <mark>SV</mark> AGPHP ILG <mark>SV</mark> AGPHP	YPMMVRDFHA 289 YPMMVRDFHA 286
Arabidopsis thaliana_upper (M23872)	FR <mark>mrll</mark> gaev Frmkllgaev	RGVHSGTATL R E VHSGTATL	KDATSEATRD KDATSEATRD	W <mark>VTNVETT</mark> HY WVTNVENTHY	ILG <mark>SV</mark> AGPHP ILG <mark>SV</mark> AGPHP	YPMMVRDFHA 284 YPMMVREFHK 282
Oryza sativa (AB003491) Zea mays (XP_008662907)	FRMRLLGAEV	RAVHSGTATL	KDATSEAIRD	WVTNVETTHY	ILG <mark>SV</mark> AGPHP	YPMMVREFHK 293
Sorghum bicolor (AAL73524) Physcomitrella patens (XP_001752340)	FRMRLLGAEV Frmrllgaev	RA <mark>VHS</mark> GTATL RPVNSGTATL	KDATSEALRD	WVTNVETTHY WVTNVETTHY	IL GSVAGPHP	YPMNVREFHK 290 YPMIVRDFHA 282
Consensus	FRMRLLGAEV	RPVHSGTATL	KDATSEAIRD	WVTNVETTHY	ILGSVAGPHP	YPMMVRDFHA
100% Conservation						
0%		320		340		360
Synechocystis sp. (WP_010874061.1)	VIGQETROQA		LLACVGGG <mark>SN</mark>	I AMGLFYDF	IDEPAVRL IG	IEAAGESIVS 282
Mcrocystis aeruginosa (NC_010296)	VIG <mark>qetrqqs</mark> Vigaetrqqc	LEKWGGLPDI LEKWGGLPDI	LLA <mark>CV</mark> GGG <mark>SN</mark> LLACVGGG <mark>SN</mark>	AMGLFHEF AMGLFHEF	VKEASVRLIG VEEPQVRLIG	VEAAGESTAS 285 VEAAGQGLDT 281
nechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1)	VIGAETROOC VIG <mark>RETRVO</mark> C	LEKWGGLPDI LERLG <mark>R</mark> LPDV		A GLEHDE	L DERAVRLVG	IEAAGEGIET 282
Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC 024005)	CIGQETRAQA MIGKEVREQA	MEKWGGLPDI MEKWGGKPDI	LVACVGGG <mark>SN</mark> LIACVGGG <mark>SN</mark>	AMGLEHEE AMGLEHEE	INDESVRIIG VNDEDVRLIG	VEAGGEGIAP 305 VEAGGEGINL 345
nydomonas reinhardtii (XM_001703345)	VIG <mark>RETKVQ</mark> A	QEKWGGLPDI	V <mark>M</mark> A <mark>C</mark> VGGG <mark>SN</mark>	ATGIF <mark>NE</mark> F	IN <mark>DTS</mark> V <mark>R</mark> LIG	VEAGGEGVNT 320
Bacilius mycoldes (AJH19322) Thermus thermophilus (YP_144361)	VIG <mark>KETKKQ</mark> Y VIG EEV KRQS	EALEG <mark>klpe</mark> a L elf grlpda	VVA <mark>CI</mark> GGG <mark>SN</mark> LIAAVGGG <mark>SN</mark>	AMGMEYPE A <mark>I</mark> GLEAPEAY	VHDEEVALYG LPEGRPKLIG	VEAAGKGVHT 268 VEAAGEGLST 288
Pseudomonas amygdali (KKY50726)	I IG <mark>KETKE</mark> QM	QEKEGRLPDS	L I A <mark>C</mark> VGGG <mark>SN</mark>	A <mark>M</mark> GLFHPF	L <mark>DDASVE</mark> IIG	VEAGGHGVDT 272
Pseudomonas fluorescens (AJZ93490) Bradyrhizobium elkanii (WP_016847382)	VIG <mark>KETRD</mark> QL IIGHETRKQM	Q AQEGRLPDS QEAEGRLPDS	LVACIGGG <mark>SN</mark> LIACIGGG <mark>SN</mark>	AMGLEHPE AMGLEHPE	LDDQSVEIIG LDDPLIEIFG	VEAAGHGIET 274 VEAAGHGL - T 274
Medicago truncatula (AES70297)			L V A <mark>C</mark> V G G G <mark>S N</mark>	AIGLEHEE	IDDKD VRLIG	VEASGLGLES 284
0	VIG <mark>KETRKQ</mark> A					
Glycine max (XP_003519001) Camptotheca acuminata (AAB97526)	VIGKETRKQA VIGKETRKQA VIGKETRKQA	LEKWGGKPDI LEKWGGKPDI LEKWGGKPDV	LIACVGGG <mark>SN</mark> LVACVGGG <mark>SN</mark>	AMGLFNEF AMGLFHEF		VEAAGFGLDS 351 VEAAGFGLDS 338
Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484)	VIGKETRKQA VIGKETRKQA VIGKETRKQA	LEKWGGKPDI LEKWGGKPDV MEKWGGKPDV	LTACVGGG <mark>SN</mark> LVACVGGGSN LVACVGGG <mark>S</mark> N	AMG <mark>LFNEF</mark> AMGLFHEF AMGLFY <mark>E</mark> F	VDDKDVRLIG VDDKDVRMIG VDDEDVRLIG	VEAAGFGLDS 338 VEAAGFGLDS 330
Camptotheca acuminata (AAB97526)	VIG <mark>KETRKQ</mark> A VIG <mark>KETRKQ</mark> A	LEKWGG <mark>KPDI</mark> LEKWGG <mark>KPDV</mark>	LTA <mark>CV</mark> GGG <mark>SN</mark> LVA <mark>C</mark> VGGG <mark>SN</mark>	A <mark>M</mark> G <mark>LFN</mark> EF AMGLFHEF	VDDKDVRLIG VDDKDVRMIG	VEAAGFGLDS 338
Camptotheca acuminata (AAB97528) Vitis vinifera (XP_002281484) Arabidopsis thaliana 0wer (M81620) Brassica napus (XP_013706880) Arabidopsis thaliana_upper (M23872)	VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRRQA VIGKETRKQA	LEKWGGKPDT LEKWGGKPDV MEKWGGKPDV MEKWGGKPDV MEKWGGKPDV LEKWGGKPDV	L ACVGGGSN L VACVGGGSN L VACVGGGSN L VACVGGGSN L VACVGGGSN L VACVGGGSN L VACVGGGSN	AMGLFNEF AMGLFHEF AMGLFYEF AMGLFHEF AMGLFHEF AMGLFHEF AMGLFHEF	VDDKDVRLIG VDDKDVRMIG VDDEDVRLIG VDDEVRMIG VDDTEVRMIG VDDTEVRMIG VNDTEVRMIG	VEAAGFGLDS 338 VEAAGFGLDS 330 VEAAGFGLDS 347 VEAAGFGLDS 344 VEAAGFGLDS 342
Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484) Arabidopsis thaliana_lower (M81620) Brassica napus (XP_013706880)	VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRRQA	LEKWGGKPDT LEKWGGKPDV MEKWGGKPDV MEKWGGKPDV MEKWGGKPDV	L LACVGGGSN L VACVGGGSN L VACVGGGSN L VACVGGGSN L VACVGGGSN	AMGLENEE AMGLEHEE AMGLEYEE AMGLEHEE AMGLEHEE	VDDKDVRLIG VDDKDVRMIG VDDEDVRLIG VDDTEVRMIG VDDTEVRMIG	VEAAGFGLDS 338 VEAAGFGLDS 330 VEAAGFGLDS 347 VEAAGFGLDS 344
Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484) Arabidopsis thaliana lower (M81620) Brassica napus (XP_013706880) Arabidopsis thaliana_upper (M23872) Oryza sativa (AB003491) Zea mays (XP_008662907) Sorghum bicolor (AAL73524)	VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRRQA VIGKETRRQA VIGKETRRQA	LEKWGGKPDV MEKWGGKPDV MEKWGGKPDV MEKWGGKPDV LEKWGGKPDV LEKWGGKPDV MDKWGGKPDV MDKWGGKPDV	L ACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN	AMGLENEF F - AMGLEYEF - - AMGLEYEF - - AMGLEHEF - -		VE AAG GLDS 338 VE AAG GLDS 330 VE AAG GLDS 347 VE AAG GLDS 344 VE AAG GLDS 342 VE AAG GVDS 342 VE AAG GVDS 341 VE AAG GVDS 341 VE AAG GVDS 343
Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484) Arabidopsis thaliana Jower (M81620) Brassica napus (XP_013706880) Arabidopsis thaliana_upper (M23872) Oryza sattva (AB003491) Zea mays (XP_008662907)	VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRRQA VIGKETRKQA VIGKETRKQA	LEKWGGKPDV MEKWGGKPDV MEKWGGKPDV MEKWGGKPDV LEKWGGKPDV LEKWGGKPDV MDKWGGKPDV MDKWGGKPDV MDKWGGKPDV	L ACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN VACVGGGSN	AMGLFNEF F - AMGLFNEF - -	VDDKD VRL G VDDKD VRL G VDDED VRL G VDDE VRM G VDDTE VRM G VDDTE VRM G VDDGE VRM G VDDGE VRM G	VE AAG GLDS 338 VE AAG FGLDS 330 VE AAG FGLDS 347 VE AAG FGLDS 344 VE AAG FGLDS 342 VE AAG FGLDS 340 VE AAG FGUDS 340 VE AAG FGVDT 351 VE AAG FGVDT 348 VE AAG FGTNT 340
Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484) Arabidopsis thaliana lower (M81620) Brassica napus (XP_013706880) Arabidopsis thaliana_upper (M23872) Oryza sativa (AB003491) Zea mays (XP_008662907) Sorghum bicolor (AAL73524) Physcomitrella patens (XP_001752340)	VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRKQA VIGKETRRQA VIGKETRRQA VIGKETRRQA VIGKETRRQA VIGKETRRQA VIGKETRRQA VIGKETRRQA	LEKWGGKPDV MEKWGGKPDV MEKWGGKPDV MEKWGGKPDV LEKWGGKPDV LEKWGGKPDV MDKWGGKPDV MDKWGGKPDV MDKWGGKPDV	ACVGGGSN VACVGGGSN VACVGGGSN	AMGLENEF F - AMGLEYEF - - AMGLEYEF - - AMGLEHEF - -	VDDKDVRLG VDDKDVRMG VDDEDVRMG VDDTEVRMG VDDTEVRMG VDDTEVRMG VDDEVRMG VDDQEVRMG VEDQDVRLG VEDQDVRLG IEDEDIRLG	VE AAG GLDS 338 VE AAG FGLDS 330 VE AAG FGLDS 347 VE AAG FGLDS 344 VE AAG FGLDS 342 VE AAG FGLDS 340 VE AAG FGUDS 340 VE AAG FGVDT 351 VE AAG FGVDT 348 VE AAG FGTNT 340

Synechocystis sp. (WP_010874061 Mcrocystis aeruginosa (NC 0102 Mcrocystis aeruginosa (NC_01025 Thermosynechococcus elongatus (NP_683264 Gioeobactar violaceus (NP_925704 Ostreococcus tauri (NC_01443 Bathycoccus prasinos (NC_02400 Chlamydomonas reinhardti (XM_0017033) Bacillus mycoides (AJH1933 Thermus thermophilus (YP_14436 Beautiopenes omendali (KV56072) Pseudomonas anygdali (KK/507) Pseudomonas fluorescens (AJ2934) Bradyrhizobium elkanii (WP_0168473) Medicago truncatula (AES702) Medicago runcatula (AES7028 Giycine max (XP_00351900 Camptotheca acuminata (AAB9752 Vitis vinifera (XP_00228148 Arabidopsis thaliana_lower (M8162 Brassica napus (XP_01370688 Arabidopsis thaliana_upper (M2337 Oryza sativa (AB00348 Zea mays (XP_00866290 Sorghum bicolor (AAL735) Physcomitrella patens (XP_00175234 Consens Conservat

Synechocystis sp. (WP_010874061 Microcystis aeruginosa (NC_01029 Thermosynechococcus elongatus (NP_683264 Gloeobacter violaceus (NP_925704 Ostreococcus tauri (NC_01443 Bathycoccus prasinos (NC_0240 Chlamydomonas reinhardtii (XM_0017033 Badilus mycoides (AIH193 Themus themophilus (YP_1443) Pseudomonas amygdali (KKY507 Pseudomonas fluorescens (AJZ934 Bradyrhizobium elkanii (WP_0168473 Medicago truncatula (AES702 Glycine max (XP_0035190 Camptotheca acuminata (AAB9752 Vitis vinifera (XP_0022814 Arabidopsis thallana_lower (M816 Brassica napus (XP_0137068 Arabidopsis thaliana_upper (M238 Oryza sativa (AB0034) Zea mays (XP_0086629) Sorghum bicolor (AAL735) Physcomitrella patens (XP_00175234 Consens

Synechocystis sp. (WP_010874061 Mcrocysta seruginosa (NC_01025 echococcus elongatus (NP_683264 Gioeobacter violaceus (NP_925704 Ostreococcus tauri (NC_01443 Bathycoccus prasinos (NC_0240 Chiamydomonas reinhardili (XM_0017033 Bacilius mycoldes (AJH193 Bacillus mycoloes (ADF196) Thermus thermophilus (YP_1443) Pseudomonas amygdali (KKY507) Pseudomonas fluorescens (AJ2934) Bradymizobium elkanii (WP_0168473) Medicago truncatula (AES702) Giycine max (XP_0035190)

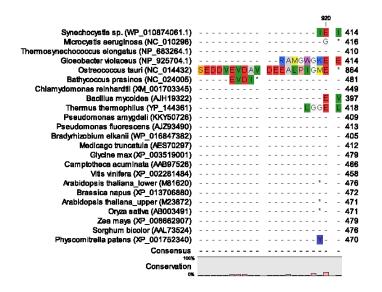
Giycine max (X2_00351900 Camptotheca acuminata (AAB9752 Vitis vinifera (X2_00228144 Arabidopsis thaliana_lower (M8162 Brassica napus (X2_01370686 Arabidopsis thaliana_upper (M2387 Oryza sativa (AB0034 Zea mays (XP_0086629 Sorghum bicolor (AAL735 Physcomitrella patens (XP_0017523

Thermosy

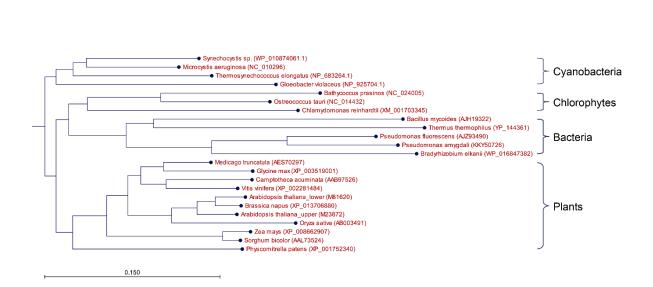
		380		400	1	420
Synechocystis sp. (WP_010874061.1)	G - <mark>K</mark> HAA <mark>TLT</mark> M	GKPGVLHG	AMSYLL <mark>QD</mark> KE	GOVTEAHSIS	AGLDYPGVGP	EHSYLKDAGR 339
Mcrocystis aeruginosa (NC_010296)	G- <mark>K</mark> HAA <mark>TLTQ</mark> G-HHAATLTK	G <mark>Q</mark> PG <mark>VLH</mark> G G EV GVLHG	A <mark>msyllqdse</mark> Amsyll qd ad	G <mark>QVTE</mark> AHSIS GQVVEAHSIS	AG <mark>LDY</mark> PGVGP AGLDYPGVGP	EHSFLKDSGR 342 EHSYLKDIGR 338
Thermosynechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP 925704.1)	G-KHAATLTA	GRAGVLHG	A <mark>msyllqd</mark> ad A <mark>msyvlqde</mark> q	GQVVEAHSIS GQVQEAHSIS	AGLDYPGVGP	EHSYLKDIGR 338 EHSYLKDIGR 339
Ostreococcus tauri (NC_014432)	G <mark>qk</mark> haa <mark>tlt</mark>	G <mark>TPGVLH</mark> G	SFSYLIQDEE	GQIVEPHSIS	AG <mark>L DY</mark> PG I GP	EHAFLK <mark>d</mark> fgr 363
Bathycoccus prasinos (NC_024005) Chlamydomonas reinhardtil (XM 001703345)	G <mark>QK</mark> HAATLTL T-KHAATLTM	G <mark>K</mark> PGVLHG GTPGVLHG	SFSYLIQDEE SYSYLLQDDD	GQIIEPHSIS GQIIDPHSIS	AGLDYPGIGP AGLDYPGIGP	EHSELKDEGR 403
Bacilius mycoides (AJH19322)	- EKHAATLTK	G <mark>SV</mark> GVLHG	SMMYLLQNEE	GQTQEAHSTS	AGLDYPGVGP	EHSLLKDIGR 325
Thermus thermophilus (YP_144361) Pseudomonas amygdali (KKY50726)	G-RHAASIGA D-KHAASING	G <mark>KR</mark> GVLHG GVPGVLHGNR	S YMYLL YDHD	GQITPAHSVS GQITDAHSIS	AGLDYPGVGP AGLDYPGIGP	EHSYYADAGV 345 Ehaflhevkr 329
Pseudomonas fluorescens (AJZ93490)	G-KHAASLNG	GVPGVLHGNR	TYLLQDGD TFLLQDDD	GOITDAHSIS	AGLDYPGIGP	EHAWLHDIGR 331
Bradyrhizobium elkanii (WP_016847382)	Q-LHAASLAG	GRPGVLHG <mark>NR</mark>	TYLLMDAD	GQIEEAHSIS	AGLDYPGIGP	EHAWLHETGR 331
Medicago truncatula (AES70297) Glycine max (XP_003519001)	G- <mark>KHAATLTK</mark> G-KHAATLTK	GEVGVLHG GEVGVLHG	AMSYLLODDD Amsylloddd	GQII <mark>E</mark> PHSIS GQIVEPHSIS	AGLDYPGVGP AGLDYPGVGP	EHSFLKDAGR 341 EHSFLKDLGR 408
Camptotheca acuminata (AAB97526)	G- <mark>K</mark> HAA <mark>TLTK</mark>	G <mark>ev</mark> gvlhg	A <mark>msyll</mark> q <mark>ddd</mark>	G <mark>qtte</mark> phsts	AGLDYPGVGP	EHSFLKDIGR 395
Vitis vinifera (XP_002281484) Arabidopsis thaliana_lower (M81620)	G-KHAATLTR G-KHAATLTK	GEVGVLHG GDVGVLHG	AMSYLLODDD Amsylloddd	GQIIEPHSIS GQIIEPHSIS	AGLDYPGVGP AGLDYPGVGP	EHSFLKDLGR 387 EHSFLKDVGR 404
Brassica napus (XP_013706880)	G-KHAATLTK	GDVGVLHG	AMSYLLQDDD	GQIIEPHSIS	AGLDYPGVGP	EHSFLKDMGR 401
Arabidopsis thaliana_upper (M23872)	G-KHAATLTK	G <mark>DVG</mark> VLHG GDVGVLHG <mark>SL</mark>	A <mark>msyllqddd</mark> Amsyllqddd	GQII <mark>E</mark> PHSIS GQVIEPHSIS	AGLDYPGVGP AGLDYPGVGP	EHSFFKDMGR 399
Oryza sativa (AB003491) Zea mays (XP_008662907)	V-KHAATLIK D-KHAATLIK	GDVGVLHG <mark>SL</mark> GQVGVLHG	AMSYLLQDDD Smsyllqddd	GQVIEPHSIS GQVIEPHSIS	AGLDYPGVGP	EHSELKDIGR 408
Sorghum bicolor (AAL73524)	D-KHAATLTK	G <mark>E</mark> VGVLHG	SMS <mark>YLLQ</mark> DD	G <mark>QVIE</mark> PHSIS	AG <mark>LDY</mark> PG <mark>V</mark> GP	EHSFLKDIGR 405
Physicomitrella patens (XP_001752340)	S-KHAATLTM G-KHAATLTK	GEVGVLHG	AMSYLLODGD Amsylloddd	GQIIEPHSIS	AGLDYPGVGP AGLDYPGVGP	EHSELKDVGR 397 EHSELKDIGR
Consensus		GEVGYLNG		Gui IEPnala	AGED IPGVGP	
Conservation or		440				480
Synechocystis sp. (WP 010874061.1)	AEYYSVTDOE	ATALORISO	LEGILPALET		CPQLKNG <mark>ER I</mark>	
Microcystis aeruginosa (NC_010296)	AEYYSVTDRE	AL <mark>E</mark> AF <mark>QRVSR</mark>	LEGIIPALET	AHALAYLETL	CPQVTG <mark>SPR</mark> I	VIN <mark>ES</mark> GRG <mark>DK</mark> 402
Thermosynechococcus elongatus (NP_683264.1) Gioeobacter violaceus (NP 925704.1)	AEYYSVTDTE AEYYSVTDSE	AVAA <mark>CVRL</mark> AQ ALAALSLVCS	LEGILPALET TEGIIPALET	AHALAYL <mark>e</mark> tl Ahafaylgil	CPOLTGOPRI ASOLHSEOTV	VINCSGRGDK 398 VLNCSGRGDK 399
Ostreococcus tauri (NC_014432)	VEYHAITDKE	ALDAFV <mark>NTSR</mark>	LEGIIPALET	SHALAYLWKL	CP G L P N G T K V	VLNCSGRGDK 423
Bathycoccus prasinos (NC_024005)	AEYYPATDQE AEYYAVTDAE	ALDAFVFTSR ALEGFOLLSK	LEGIIPALET	SHALAYLEKL Shalaylekl	CPTLPDKCKV TPTLKSGTRV	VLNESGRGDK 463
Chlamydomonas reinhardtii (XM_001703345) Bacillus mycoides (AJH19322)	VSYHSITDEE	ALEAFOLLTK	KEGIIPALES	SHAVAYALKL	AP <mark>qmkk</mark> degl	VINCSGRGDK 437
Thermus thermophilus (YP_144361)	AEYASVTDEE	ALEGE <mark>KLLAR</mark>	LEGIIPALES	AHATAYAAKV	V P E MD K D Q V V	VINLSGRODK 405
Pseudomonas amygdall (KKY50726) Pseudomonas fluorescens (AJZ93490)	VEYVSITDDE VQYTSVTDDE	ALDAFHQCCL ALDAFHKCCR	LEGIIPALET	AHALA <mark>E</mark> AMKR Ahala <mark>evek</mark> r	ATNLRDDHLM APTLPKDHLM	VVCLSGRGDK 388 VVNLSGRGDK 391
Bradyrhizobium elkanii (WP_016847382)	VKYLSATDEE	ALAAFQLLS <mark>R</mark>	LEGIIPALES	AHATA <mark>klse</mark> l	AP <mark>QR</mark> PK D HLM	VVNLSGRG <mark>DK</mark> 391
Medicago truncatula (AES70297) Glycine max (XP_003519001)	AEYYSVTDEE AEYHSITDEE	ALEAFKRVSQ ALEAFKRVSR	LEGIIPALET LEGIIPALET	SHALAYL <mark>ek</mark> l Shalaylekv	CPTLPNG <mark>TK</mark> V CPTLPNGAKV	VVNCSGRGDK 401
Camptotheca acuminata (AAB97526)	AEYYCCTDEE	ALEAFKRLS <mark>R</mark>	LEGIIPALET	SHALAFLEKL	CPTLPNGTKV	VLNCSGRGDK 455
Vitis vinifera (XP_002281484)	AEYHSITDEE	ALEAFKRLSR	LEGIIPALET	SHALAYL <mark>ek</mark> l	CPTLPNGTKV	VLNCSGRGDK 447
Arabidopsis thallana_lower (M81620) Brassica napus (XP 013706880)	AEYFSVTDEE AEYYSVTDEE	ALEAFKRVSR ALEAFKRVSR	LEGIIPALET LEGIIPALET	SHALAHL <mark>ek</mark> l Shalahl ek l	CPTLPDGARV CPTLPDGARV	VLNESGRGDK 464 VLNESGRGDK 461
Arabidopsis thaliana_upper (M23872)	AEYYSITDEE	ALEAFKRVSR	LEGIIPALET	SHALAYL <mark>ek</mark> l	CPTLSDGTRV	VLNFSGRGDK 459
Oryza sativa (AB003491) Zea mays (XP_008662907)	AEYDSATDEE AEYDSVTDQE	ALAGEKRVSR ALDAEKRVSR	LEGIIPALET LEGIIPALET	SHALAYL <mark>ek</mark> l Shalaylekl	CPTLFDGTRV CPTLADGVRV	VFNESGRGDK 459
Sorghum bicolor (AAL73524)	AEYDSVTDQE	ALDAFKRV <mark>S</mark> R	LEGIIPALET	SHALAYL <mark>ek</mark> l	CPTLP <mark>D</mark> G <mark>VR</mark> V	VV <mark>NCS</mark> GRG <mark>DK</mark> 465
Physcomitrella patens (XP_001752340)	AEYESVIDDE	ALEAFORLSK	LEGIIPALET	SHALAFLETL	CPTLEDGTRV CPTLPDGTRV	VLNCSGRGDK 457
Consensus 100% Conservation	AEYYSVTDEE	ALEAFKRVSR		SHALAYLEKL	CPILPDGIRV	VLNCSGRGDK
CONSTANTO		500		520		64 0
Synechocystis sp. (WP_010874061.1)						398
Mcrocystis aeruginosa (NC_010296) Thermosynechococcus elongatus (NP 683264.1)						402
Gloeobacter violaceus (NP_925704.1)						
Ostreococcus tauri (NC_014432)	XXXXXXX <mark>TTS</mark>	MVGMKSVELL		AP <mark>V</mark> G <mark>D</mark> G <mark>S</mark> AA <mark>S</mark>	A <mark>DDT</mark> A <mark>K</mark> A <mark>VV</mark> A	SWERRNREVP 483
Bathycoccus prasinos (NC_024005) Chiamydomonas reinhardiil (XM 001703345)						463
Bacilius mycoldes (AJH19322)						385
Thermus thermophilus (YP_144361) Pseudomonas amygdali (KKY50726)						405
Pseudomonas fluorescens (AJZ93490)						391
Bradyrhizobium elkanii (WP_016847382)						391
Medicago truncatula (AES70297) Glycine max (XP_003519001)						
Camptotheca acuminata (AAB97526)						455
Vitis vinifera (XP_002281484) Arabidopsis thaliana lower (M81620)						
Brassica napus (XP_013706880)						461
Arabidopsis thaliana_upper (M23872)						458
Oryza sativa (AB003491) Zea mays (XP 008662907)						458
Sorghum bicolor (AAL73524)						465
Physcomitrella patens (XP_001752340)						457
Consensus						
Conservation						

Synechocystis sp. (WP_010874061.1)		560 		580 		•
Mcrocystis aeruginosa (NC_010296)						
osynechococcus elongatus (NP_683264.1)						
Gloeobacter violaceus (NP_925704.1)						
Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005)		NSDA <mark>REL</mark> ACA		GEVWESMASW		
lamydomonas reinhardtil (XM_001703345)						
Bacilius mycoides (AJH19322)						
Thermus thermophilus (YP_144361) Pseudomonas amygdali (KKY50726)						
Pseudomonas fluorescens (AJZ93490)						
Bradyrhizobium elkanii (WP_016847382)						
Medicago truncatula (AES70297)						
Giycine max (XP_003519001) Camptotheca acuminata (AAB97526)						
Vitis vinifera (XP_002281484)						
Arabidopsis thaliana_lower (M81620)						
Brassica napus (XP_013706880)						
Arabidopsis thaliana_upper (M23872) Oryza sativa (AB003491)						
Zea mays (XP_008662907)						
Sorghum bicolor (AAL73524)						
Physcomitrella patens (XP_001752340)						
Conservation						
0%		620		840		
Synechocystis sp. (WP_010874061.1)						
Mcrocystis aeruginosa (NC_010296)						
osynechococcus elongatus (NP_683264.1)						
Gioeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432)	MMONESMIAR		AFDRVPFAHG			
Bathycoccus prasinos (NC 024005)					SVVATAL VISA	
lamydomonas reinhardtii (XM_001703345)						
Bacillus mycoides (AJH19322)						
Thermus thermophilus (YP_144361)						
Pseudomonas amygdall (KKY50726) Pseudomonas fluorescens (AJZ93490)						
Bradyrhizobium elkanii (WP_016847382)						
Medicago truncatula (AES70297)						
Glycine max (XP_003519001)						
Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484)						
Arabidopsis thallana_lower (M81620)						
Brassica napus (XP_013706880)						
Arabidopsis thaliana_upper (M23872)						
Oryza sativa (AB003491)						
Zea mays (XP_008662907) Sorghum bicolor (AAL73524)						
Physcomitrella patens (XP_001752340)						
Consensus						
100% Conservation						
0%		680		700		
				1		
	DVQTVA-					
Synechocystis sp. (WP_010874061.1)	<mark>D VQTV</mark> A - <mark>D VQSV</mark> A -					
Synechocystis sp. (WP_010874061.1) Mcrocystis aeruginosa (NC_010296) synechococcus elongatus (NP_683264.1)	<mark>DVQSV</mark> A- DVETI G-					
Synechocystis sp. (WP_010874061.1) Mcrocystis aeruginosa (NC_010286) synechococcus elongatus (NP_683264.1) Gioeobacter violaceus (NP_925704.1)	DVQSV A- DVETI G- DMGTV A-					
Synechocystis sp. (WP_010874061.1) Mcrocystis aeruginosa (NC_010296) synechococcus elongatus (NP_683264.1) Giocobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432)	DVQSV A- DVETI G- DMGTV A-					
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010296) synechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005)	DVQSVA- DVETIG- DMGTVA- DGVDDTQVAV			KIGATDGGGL		
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010286) synechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardtii (XM_001703345) Bacillus mycoldes (AlH19322)			VDVTNVTALK	KIGATDGGG	RGAYWRLMTG	G <mark>SVD</mark> GGGGA
Synechocystis sp. (WP_010874061.1) Mcrocystis aeruginosa (NC_010296) synechococcus elongatus (NP_683264.1) Gioeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardill (XM_001703345) Bacilius mycoldes (ALH19322) Themus thermophilus (YP_144361)	D V Q S V A D V Q T I G D MG T V A D V T Q A A 		VOVTNVTALK	KIGATDGGG	RGAYWR MTG	G <mark>SVD</mark> GGGGA
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010286) synechococcus elongatus (NP_68286.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardtil (XM_001703345) Bacilius mycoldes (AJH19322) Thermus thermophilus (YP_144361) Pseudomonas argydali (KKY50728)	DVGVA DVGVA DVGVA DVGVA DVTQAA DVNA DVEVR DVTQVA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA 			GATOGGG	RGAYWR MTG	G <mark>SWD</mark> GGGGA
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010286) synechococcus elongatus (NP_683264.1) Giocobacter violaccus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardti (XM_00170345) Bacilius mycoides (AJH19322) Thermus thermophilus (YP_144381) Pseudomonas amygdali (KKY50728) Pseudomonas fluorescens (AJ283490)	DVQSVA DVGTVG- GVDTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQVA DVTVA DVTVA DVTVA DVTVA			GATDGGG	RGAYWR MTG	G <mark>SVD</mark> GGGGA
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010286) synechococcus elongatus (NP_68286.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardtil (XM_001703345) Bacilius mycoldes (AJH19322) Thermus thermophilus (YP_144361) Pseudomonas argydali (KKY50728)	DVGVA DVGVA DVGVA DVGVA DVTQAA DVNA DVEVR DVTQVA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA DVTQAA 			KI CAT GGG	RGA WWR I MTG	G <mark>SVD</mark> GGGGA
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010286) synechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardtil (XM_001703345) Bacilius mycoldes (AIH19322) Thermus thermophilus (YP_144381) Pseudomonas amygdali (KKY50726) Pseudomonas filuorescens (AJ293490) Bradyrhizoblum elkanii (WP_016847382) Medicago truncatula (AES70297) Glycine max (XP_003519001)				K GATDGGG	R GAYWR MT G	G <mark>SVI</mark> GGGGA
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010296) synechococcus elongatus (NP_683264.1) Gloeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardtil (XM_00170345) Bacillus mycoides (AJH19322) Thermus thermophilus (YP_144361) Pssudomonas amygdali (KKY50726) Pseudomonas fluorescens (AJ283490) Bradyrhizoblum elkanil (WP_016847382) Mødicago truncatula (AES70297) Gilyche max (XP_003519001) Camptotheca acuminata (AAB97526)				KI CAT GGG	R CA WWR MT G	GSNDGGG
Synechocystis sp. (WP_010874061.1) Microcystis aeruginosa (NC_010286) synechococcus elongatus (NP_682864.1) Gioeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinoe (NC_024005) amydomonas reinhardtil (XM_001703345) Bactilus mycoldes (AIH19322) Thermus thermophilus (YP_144361) Pseudomonas amygdali (KKY50726) Pseudomonas fluorescens (AI283490) Bradythizoblum elikanil (WP_016847382) Medicago truncatula (AES70297) Glychne max (XP_003519001) Camptotheca acuminata (AAB97526) Vitis vinifera (XP_002281484)		MARC VOMANH Me - Haaa - Ekt Haaa - Ekt Ka		K GAT GGG	RGA MWR MI G	GSVDGGGG
Synechocystis sp. (WP_010874061.1) Mcrocystis aeruginosa (NC_010286) synechococcus elongatus (NP_683264.1) Giocobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardtil (XM_001703345) Bacilius mycoldes (AIH19322) Thermus thermophilus (YP_144381) Pseudomonas amygdali (KKY50726) Pseudomonas filorescens (AJ293490) Bradynhizoblum elkanil (WP_016847382) Medicago truncatula (AES70297) Giyche max (XP_003519001) Camptotheca acuminata (AAB97526) Vftis vinifera (XP_002281484) Arabidopsis thaliana_lower (M81620)		MARC VOMANH Me - Haaa - Ekt Haaa - Ekt Ka		K GAT GGG	R GAYWR MT G	GSVDGGGG
Synechocystis sp. (WP_010874061.1) Mcrocystis aeruginosa (NC_010286) synechococcus elongatus (NP_68286.1) Gioeobacter violaceus (NP_925704.1) Ostreococcus tauri (NC_014432) Bathycoccus prasinos (NC_024005) amydomonas reinhardill (XM_001703345) Bacillus mycoldes (AJH19322) Thermus thermophilus (YP_144361) Pseudomonas fluorescens (AJ283490) Bradyhizoblum elikanil (WP_016847382) Medicago truncatula (AES70297) Giycine max (XP_003519001) Camplotheca acuminata (AAB97528) Vitis vinitera (XP_002281484)		MARCINDMANH Me Haaaa - Ekt Me Soort Ka		KI CATOGGG	R GA YWR MT G	GSNDGGG
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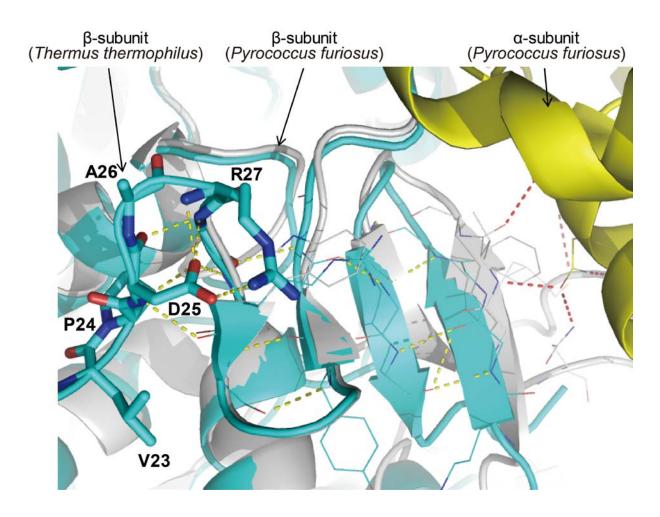
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Pseudomonas fluorescens (AJZ93490)						
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Arabidopsis thaliana lower (M81620)						
Brassica napus (XP_013706880)					k	YLE-V
Arabidopsis thaliana upper (M23872)					<mark>K</mark>	YLD - V
Oriza sativa (AB003491)					<mark>K</mark>	SID-V
Zea mays (XP 008662907)					K	YLD - V
Sorghum bicolor (AAL73524)					<mark>K</mark>	YLD - V
Physcomitrella patens (XP_001752340)					G	YL <mark>K</mark> G <mark>K</mark>
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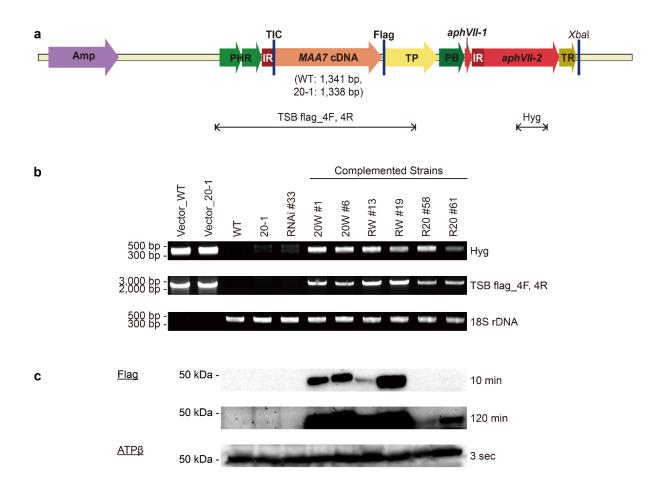
Supplementary Fig. 1. Alignment of the full-length TSB amino acid sequences from organisms representing three kingdoms.



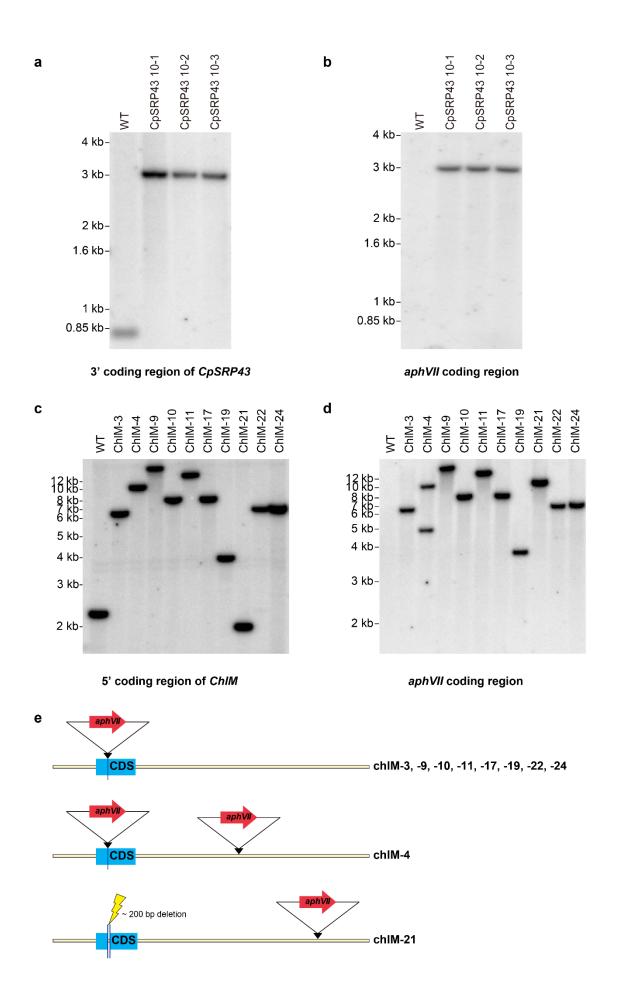
Supplementary Fig. 2. Phylogenetic tree of the TSB amino acid sequences.



Supplementary Fig. 3. Crystal structures of tryptophan synthases and the region corresponding to the RPDAN motif. The α and the β subunits of the tryptophan synthase from *Pyrococcus furiosus* (PDB CODE 1WDW) is shown as a cartoon diagram with yellow and gray colors, respectively. The β subunit from *Thermus thermophilus* (PDB CODE 1X1Q) is superposed with the corresponding subunit of the tryptophan synthase from *P. furiosus*, and is shown in cyan. The β subunit of the tryptophan synthase from *T. thermophilus* has an LPDAR (stick model) sequence at the position of the RPDAN motif of *C. reinhardtii*. The hydrogen bonds forming the β -sheet are shown as dotted yellow lines, and those forming the interface between the two subunits are shown as dotted red lines.



Supplementary Fig. 4. Select complemented strains of MAA7 mutants. (a) Schematic diagram of the vector used for complementation. Abbreviations: PHR, HSP70A-RBCS2 promoter; IR, RBCS2 intron; TIC, translation initiation context; TP, PsaD terminator; PB, beta2-tub promoter; and TR, RBCS2 terminator. (b) PCR confirmation of the integration of vector sequences into the complemented strains. The PCR amplicons for the hygromycin resistance region and 18S rDNA were 387 bp and 380 bp, respectively, while those encompassing the MAA7 cDNA and Flag tag were 2,477 bp for WT and 2,474 bp for MAA7 20-1. (c) Western blotting of FLAG-tagged MAA7 proteins from complemented strains. The expected sizes of FLAG-tagged MAA7 were 49.6 kDa for WT and 49.5 kDa for MAA7 20-1. ATPβ (the beta subunit of ATP synthase; 53.9 kDa) was used as a loading control.



Supplementary Fig. 5. Southern blot analyses of the CpSRP43 and ChIM mutants. Genomic DNAs of the *CpSRP43* mutants were digested with *Pst*I and *Nco*I and subjected to Southern blotting with probes against the 3' coding region of *CpSRP43* (**a**) and the *aphVII* coding region (**b**). Genomic DNA of *ChIM* mutants was digested by *Nco*I and subjected to Southern blotting with probes against the 5' region of *ChIM* (**c**) and the *aphVII* coding region (**d**). Main classes of knock-in mutations in the ChIM locus were indicated in schematic drawing (**e**).

Supplementary Table 1. The primers used in our CRISPR/Cas9 systems.

Gene	Primer	Sequence 5'-3'	Purpose	Size (bp)
	M2_F	CAACGTTTGCCGTTGGTATG	Amplifying Cas9 cut site of	702
	M2_R	TTCAGGATAGCCTCCATCTCC	MAA7 (in vitro)	783
	I202TSB_3F I202TSB_3R	GTCGCCGTTTCCATTTGCAGGATGGCCATG GAAGCTTGATAACAATGAACATCACTGCCCC TACTC TGATACAGGCGGTCCAATCTGAATTGGGCT GCAGGAATTCGATTCACTTGTCGTCGTCGT CCTTGTAGTCAGGGTTGATGTACTTCATGGC	Vector construction for complementation of MAA7	1,455
MAA7	<u> </u>	GTT		
	aph 72_L aph 72_R	CCCCCATTCCGAGGTCTTCC AAGGTGAAGGCGAGCAGTTC	Confirmation of complemented MAA7 strains (Hygromycin resistant)	387
	TSB flag_4F	GCGCAATTAACCCTCACT	Confirmation of complemented	
	0-	CAAACTCTCACATGGCCT	MAA7 strains (transgenic MAA gene)	_2,477 for WT 72,474 for <i>MAA7</i> 20-1
	SR6 SR9	GTCAGAGGTGAAATTCTTGG AACTAAGAACGGCATGCAC	Confirmation of complemented MAA7 strains (18S rDNA)	380
S	SRP43_2F SRP43_2R	TATTAGGGCATCGACAGG TGGAAGAATTGGAATGGG	Amplifying Cas9 cut site of CpSRP43 (in vitro)	795
	SRP43_1F PRR2	CAAATACCATCGGCGTGTGG ACTGACCCACAGCTGTCCTAAGCC	Region A	509
CpSRP43 PRR hpt15 aph7 SRP- aph7 aph7 SRP- SRP- SRP- SRP-	PRR2_RC hpt1579_R	GGCTTAGGACAGCTGTGGGTCAGT TACCGCTTCAGCACTTGAGA	Region B	1,662
	aph72_L SRP43_1R	CCCCCATTCCGAGGTCTTCC GCGACACCTCGTGAAGCATA	Region C	1,089
	• –	TGGTGCAACTGCATCTCAAC CCAGAGGAACTGCGCCAGTTCCT	Southern blot of aphVII probe	203
	SRP43_4F SRP43_4R	AAATGGAAGGTGAGAGCTGG GTCAGATCCGTCCTGTGGAA	Southern blot of <i>CpSRP43</i> probe	305
	CrChIM_F1 CrChIM_R3	GTGTTGCAGAGGTGTCTCAGGTTGCCAACA CCGTCTCGCCGTAGATCTTGCGCCA	Amplifying Cas9 cut site of ChIM (in vitro)	1,010
	aph7_1471F aph7_1114R		Hygromycin resistant	203
ChIM	CrChIM_F aph7_1471F	TCGCTGCGTGGCGGAAGCACTA	Region D in ChIM-3, 22, 24	2,000 for ChIM-3 1,700 for ChIM-22 1,800 for ChIM-24
	CrChIM-F1 psaD_ter_R	GGTCTTCTCAATGGGTGTGGCACGT	Region D in ChIM-9	500
	CrChIM_F1 aph7_1471F		Region D in ChIM-10, 17	500
	CrchIM_F3 aph7_R2	GCATGGCAAGCTGGCGTCAGCA TTCCGGTCGGTCGTGCCGTCCAT	Region D in ChIM-19	650
	• – –	CCTGTGGCTAATTGACCGTG GCACTCCTGCACCCTTCTCACCGCT	Region E in ChIM-3, 22, 24	400 for ChIM-3 1,000 for ChIM-22, 24
	aph7_1471F CrChIM_R1		Region E in ChIM-9, 19	700 for ChIM-9 500 for ChIM-19
	CrChIM_F1 CrChIM_R2	CCGAGGTAAAAGGCTTGGTGTTTAGGCA	Southern blot of ChIM probe	257
		ATGCAGTGCCTAAACACCAAGCCT CCGTCTCGCCGTAGATCTTGCGCCA	RT-PCR (<i>ChIM</i>)	361
	IDA5_F IDA5_R	ATCGTGCGCGACATCAAGGA TACTCGGACTTGGCGATCCA	RT-PCR (loading control)	464