

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

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	20	40	60	
<i>Synechocystis</i> sp. (WP_010874061.1)	MNITSPISAP	SH		12
<i>Microcystis aeruginosa</i> (NC_010296)	MTITPIYAA	SAGTAT		15
<i>Thermosynechococcus elongatus</i> (NP_683264.1)	MTVA-PSAA	SAAA		14
<i>Gloeobacter violaceus</i> (NP_925704.1)	MSFTIQTYT	TAPQFGA		19
<i>Ostreococcus tauri</i> (NC_014432)	MGVKKG	-RPTAAPGRG	AT	32
<i>Bathycoccus prasinos</i> (NC_024005)	MATTSASFLS	ARSSASARG	KTCTSKSSSS	20
<i>Chlamydomonas reinhardtii</i> (XM_001703345)	MNITAPTRLD	SRPVASAR	SSRITSEQTH	63
<i>Bacillus mycoloides</i> (AJH19322)	MNVA			4
<i>Thermus thermophilus</i> (YP_144361)	MGVYARGAF	RERSM		16
<i>Pseudomonas amygdali</i> (KKY50726)	MTQ--TNFR	SG		9
<i>Pseudomonas fluorescens</i> (AJZ93490)	MTQSGTDLR	HG		11
<i>Bradyrhizobium elkanii</i> (WP_016847382)	MNPNLPSNFR	SG		12
<i>Medicago truncatula</i> (AES70297)	MENHNNDTG	SVQS		14
<i>Glycine max</i> (XP_003519001)	MAAS--IP	TGNSSRYLP	IPREPHPHFS	57
<i>Camptotheca acuminata</i> (AAB97526)	MAAYTN-PAC	RINTSA--FP	GPYRPNPS	51
<i>Vitis vinifera</i> (XP_002281484)	MAASAKTPIS	RTQTS--	WPKYSSSP	49
<i>Arabidopsis thaliana_lower</i> (M81620)	MATASTAAT	RPSVSA--	SSELTH--LR	52
<i>Brassica napus</i> (XP_013706880)	MATSGTAST	RPSVSA--	SSRLTH--LR	49
<i>Arabidopsis thaliana_upper</i> (M23872)	MAASGTSAT	RASVSSAPSS	SSQLTH--K	54
<i>Oryza sativa</i> (AB003491)	MAQASAQI-	-GSGSAQYQ	SKQTKNFTLA	52
<i>Zea mays</i> (XP_008662907)	MATIAAAR	SPTRAAAPG-	-PAAPAQRRA	54
<i>Sorghum bicolor</i> (AAL73524)	MA--AATLR	SPTRAAAPAG	PAAAPTQQR	52
<i>Physcomitrella patens</i> (XP_001752340)	MAAA--	-PTQATGCTA	SQGVVRKPSA	47
Consensus	MAXT-XA-XF	SXS-AS---		



	60	100	120	
<i>Synechocystis</i> sp. (WP_010874061.1)		QYP	DALGRFGN-Y	GGKYVPETLM
<i>Microcystis aeruginosa</i> (NC_010296)		QYP	DAFGRFGR-Y	GGKYVPETLM
<i>Thermosynechococcus elongatus</i> (NP_683264.1)		P	DARGRFR-Y	GGKYVPETLM
<i>Gloeobacter violaceus</i> (NP_925704.1)		P	DSRGRFR-Y	GGQYVPETLM
<i>Ostreococcus tauri</i> (NC_014432)	SK--EQ	P	DAAGRGR-Y	GGKYVPETLM
<i>Bathycoccus prasinos</i> (NC_024005)	MTPPPQQRVF	T	RDVLRP	DENGRYGGY
<i>Chlamydomonas reinhardtii</i> (XM_001703345)	WAPRWASRKS	TVR	AAVAAP	ERPIIDYGRP
<i>Bacillus mycoloides</i> (AJH19322)				Y
<i>Thermus thermophilus</i> (YP_144361)				LPDFP
<i>Pseudomonas amygdali</i> (KKY50726)				ANGLRF-SF
<i>Pseudomonas fluorescens</i> (AJZ93490)				ANGLRF-AF
<i>Bradyrhizobium elkanii</i> (WP_016847382)				DERGHFG-L
<i>Medicago truncatula</i> (AES70297)				DSFGRFGR-F
<i>Glycine max</i> (XP_003519001)	TKDPYKSVVP	EEHHSK-MDN	GSVL--QRP	DSFGRFGR-F
<i>Camptotheca acuminata</i> (AAB97526)	AREMEKERS	EREPDV--		DSFGRFGR-F
<i>Vitis vinifera</i> (XP_002281484)				DSFGRFGR-F
<i>Arabidopsis thaliana_lower</i> (M81620)	AKDPAAYMA	DSEKKAAGS	DPTM--WQRP	DSFGRFGR-F
<i>Brassica napus</i> (XP_013706880)	AKDPTFLMA	EAEKTKAAGS	DPTL--WQRP	DSFGRFGR-F
<i>Arabidopsis thaliana_upper</i> (M23872)	AKDPPVLLMA	-----AGS	DPAL--WQRP	DSFGRFGR-F
<i>Oryza sativa</i> (AB003491)	AKDMHVLMA	-----AGS	DPTL--WQGP	DSFGRFGR-F
<i>Zea mays</i> (XP_008662907)	QPAKAAIAEA	ASPAVEMGN	GAAAPGQRP	DAMGRFGR-F
<i>Sorghum bicolor</i> (AAL73524)	QPAKAAIAEA	ASPAVEMGN	GAAVAGMQR	DAMGRFGR-F
<i>Physcomitrella patens</i> (XP_001752340)	ARDAT--A	KDVTTRDQDD	LRTYKGERP	DSFGRFGR-F
Consensus				-QRP DXFGRFGK-F GGKYVPETLM PALXELEXAF



	140	160	180	
<i>Synechocystis</i> sp. (WP_010874061.1)	YRRAEESFQ	EEAGLKDY	VGRSSPLYFA	ERLSAHYARF
<i>Microcystis aeruginosa</i> (NC_010296)	NQYQDDPDFQ	EEENQLLKDY	VGRPSPLYFA	ERLTAHYAKA
<i>Thermosynechococcus elongatus</i> (NP_683264.1)	AHYRQDPDFQ	AEQLQLLDY	VGRPSPLYFA	ERLSAHYAH-
<i>Gloeobacter violaceus</i> (NP_925704.1)	NQYRHDPQFL	AEFAGHLRDF	VGRPTPLYFA	ERLTAHWY--
<i>Ostreococcus tauri</i> (NC_014432)	EAKTDAAAFI	EEFKSVLKDY	VGRANPLYFA	ERLSEAFKDA
<i>Bathycoccus prasinos</i> (NC_024005)	EKLKTDPAFI	EEFKSVLKDY	VGRANPLYFA	ERLSEAFKDA
<i>Chlamydomonas reinhardtii</i> (XM_001703345)	NEALADPAFK	AEMEAALKDY	VGRETPLYHA	ERLSAHYKTA
<i>Bacillus mycoloides</i> (AJH19322)	KEAMDDPAFK	KELDHYLQTY	VGRETPLYFA	ERNMTKYC--
<i>Thermus thermophilus</i> (YP_144361)	REAKKDPAPF	EELDHYLRQF	AGRPTPLYHA	KRLSEYVW--
<i>Pseudomonas amygdali</i> (KKY50726)	EAAKADPEFA	KEMAYFORDY	VGRPNPLYFA	ERLTERC---
<i>Pseudomonas fluorescens</i> (AJZ93490)	EAAKEDPAFK	KEEAYFORDY	VGRPNPLYFA	ERLTERC---
<i>Bradyrhizobium elkanii</i> (WP_016847382)	ADAKADPAFK	AEMNLYLKDY	VGRPSPLYFA	ERLTERH---
<i>Medicago truncatula</i> (AES70297)	HSLASDEDFQ	KEEAGVLDKY	VGRESPLYFA	ERLTERHYKRG
<i>Glycine max</i> (XP_003519001)	YSLSADEEFQ	KEEAGVLDKY	VGRESPLYFA	ERLTERHYKRP
<i>Camptotheca acuminata</i> (AAB97526)	RSLSGDQVFQ	KEEDGTLKDY	VGRESPLYFA	ERLTERHYKRP
<i>Vitis vinifera</i> (XP_002281484)	RALSGDVEFQ	KEEDGTLKDY	VGRESPLYFA	ERLTERHYKRP
<i>Arabidopsis thaliana_lower</i> (M81620)	YSLATDDEDQ	RELAELKDY	VGRESPLYFA	ERLTERHYRR
<i>Brassica napus</i> (XP_013706880)	YSLATDDEDQ	RELAELKDY	VGRESPLYFA	ERLTERHYRR
<i>Arabidopsis thaliana_upper</i> (M23872)	YALATDDEDQ	RELAELKDY	VGRESPLYFA	ERLTERHYRR
<i>Oryza sativa</i> (AB003491)	KKLATDDEDQ	RELAELKDY	VGRESPLYFA	ESLTERHYRR
<i>Zea mays</i> (XP_008662907)	HALATDDEFQ	KEEDGTLKDY	VGRESPLYFA	ERLTERHYKRA
<i>Sorghum bicolor</i> (AAL73524)	HALATDDEFQ	KEEDGTLKDY	VGRESPLYFA	ERLTERHYKRA
<i>Physcomitrella patens</i> (XP_001752340)	RATIKDPEFQ	KEELNDYLDY	VGRETPLYFA	ERLTERHYKRP
Consensus	YALATDPXFQ	KELAGILKDY	VGRESPLYFA	ERLTERHYKR-DGXGPLYLK REDLNHTGAH



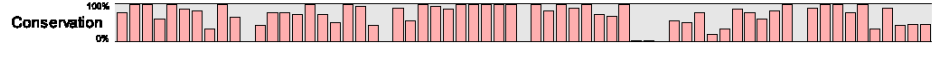
	200	220	240	
<i>Synechocystis</i> sp. (WP_010874081.1)	K I N N A I G Q V L L A K R M G K K R I A E T G A G Q H G V A T A T V C A R F G L E C I Y M G A Q D M E R Q A L N V	164		
<i>Microcystis aeruginosa</i> (NC_010296)	K I N N A I G Q V L L A K R M G K K R I A E T G A G Q H G V A T A T V C A R F G L E C V Y M G I H D M E R Q E L N V	167		
<i>Thermosynechococcus elongatus</i> (NP_683264.1)	K I N N A I G Q V L L A K R M G K Q R I A E T G A G Q H G V A T A T V C A R F G L Q C V Y M G Y Q D M E R Q R L N V	163		
<i>Gloeobacter violaceus</i> (NP_925704.1)	K I N N A I G Q A L L A R M G K R R I A E T G A G Q H G V A T A T V C A R F G L E C I Y M G Y H D I E R Q K L N V	164		
<i>Ostreococcus tauri</i> (NC_014432)	K I N N A I G Q A L L A K R M G K K R I A E T G A G Q H G V A T A T V C A R F G L E C I Y M G A A D M E R Q K L N V	187		
<i>Bathycoccus prasinos</i> (NC_024005)	K I N N A I G Q A I L A K R M G K K R I A E T G A G Q H G V A T A T V C A R F G L E C I Y M G A A D M E R Q K L N V	227		
<i>Chlamydomonas reinhardtii</i> (XM_001703345)	K I N N S I G Q A L L A K R L N K Q R I A E T G A G Q H G V A T A T V C A R Y G L K C I Y M G A K D M E R Q A L N V	202		
<i>Bacillus mycoides</i> (AJH19322)	K I N N T I G Q A L L A V R M G K K K V I A E T G A G Q H G V A T A T V C A L F G L E C V F M G E E D V R R Q A L N V	150		
<i>Thermus thermophilus</i> (YP_144361)	K I N N T I G Q A L L A R R M G K R R V I A E T G A G Q H G S V A T A A A F G L E C V Y M G E E D V R R Q A L N V	168		
<i>Pseudomonas amygdali</i> (KKY50726)	K I N N C I G Q V L L A K R M G K K R I A E T G A G M H G V A T A T V A A R F G L P C V Y M G A T D I E R Q Q A N V	154		
<i>Pseudomonas fluorescens</i> (AJZ93490)	K I N N C I G Q I L L A R R M G K K R I A E T G A G M H G V A T A T V A A R F G L Q C V Y M G T F T D I E R Q Q A N V	156		
<i>Bradyrhizobium elkanii</i> (WP_016847382)	K V N N V I G Q M V A R R M G K K R I A E T G A G Q H G V A T A T L C A R F G L E C V Y M G A V D E R E Q Q P N V	157		
<i>Medicago truncatula</i> (AES70297)	K I N N A I A Q A L L A K K L G K K R I A E T G A G Q H G V A T A T V C A R F G L E C V Y M G A L D M E R Q A L N V	166		
<i>Glycine max</i> (XP_003519001)	K I N N A I A Q A L L A K R L G K K R I A E T G A G Q H G V A T A T V C A R F G L E C I Y M G A Q D M E R Q A L N V	233		
<i>Camptotheca acuminata</i> (AAB97526)	K I N N A I A Q A L L A K R L G K K R I A E T G A G Q H G V A T A T V C A R F G L Q C V Y M G A Q D M E R Q A L N V	220		
<i>Vitis vinifera</i> (XP_002281484)	K I N N A I A Q A L L A K R L G K T R I A E T G A G Q H G V A T A T V C A R F G L Q C V Y M G A Q D M E R Q A L N V	212		
<i>Arabidopsis thaliana_lower</i> (M81620)	K I N N A I A Q A L L A K R L G K K R I A E T G A G Q H G V A T A T V C A R F G L Q C I Y M G A Q D M E R Q A L N V	229		
<i>Brassica napus</i> (XP_013706880)	K I N N A I A Q A L L A K R L G K K R I A E T G A G Q H G V A T A T V C A R F G L Q C I Y M G A Q D M E R Q A L N V	226		
<i>Arabidopsis thaliana_upper</i> (M23872)	K I N N A I A Q A L L A K R L G K K R I A E T G A G Q H G V A T A T V C A R F G L Q C I Y M G A Q D M E R Q A L N V	224		
<i>Oryza sativa</i> (AB003491)	K I N N A I A Q A L S A K R L G K K R I A E T G A G Q H G V A T A T V C A S F G L E C I Y M G A Q D M E R Q A L N V	222		
<i>Zea mays</i> (XP_008662907)	K I N N A I A Q A L L A K R L G K Q R I A E T G A G Q H G V A T A T V C A R F G L Q C I Y M G A Q D M E R Q A L N V	233		
<i>Sorghum bicolor</i> (AAL73524)	K I N N A I A Q A L L A K R L G K Q R I A E T G A G Q H G V A T A T V C A R F G L Q C I Y M G A Q D M E R Q A L N V	230		
<i>Physcomitrella patens</i> (XP_001752340)	K I N N A I A Q A L L A K R L G K K R I A E T G A G Q H G V A T A T V C A R F G L E C I Y M G A Q D M E R Q A L N V	222		



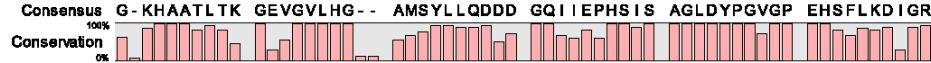
	260	280	300	
<i>Synechocystis</i> sp. (WP_010874081.1)	F R M N L L G A R V Q P V T A G T G T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H R	224		
<i>Microcystis aeruginosa</i> (NC_010296)	F R M R L L G A T V Q P V A A G T G T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	227		
<i>Thermosynechococcus elongatus</i> (NP_683264.1)	L R M R L L G A E V A P V S A G T G T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M L V R E F H A	223		
<i>Gloeobacter violaceus</i> (NP_925704.1)	Y R M K L L G A E V R P V A A G T G T I K D A T S E A I R D W V T N V E T T H Y I L G S A A G P H P Y P M V R E F Q A	224		
<i>Ostreococcus tauri</i> (NC_014432)	F R M R L L G A T V R P V A G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	247		
<i>Bathycoccus prasinos</i> (NC_024005)	F R M R L L G A T V I P R V T G T S T I K D A T S Q A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H S	287		
<i>Chlamydomonas reinhardtii</i> (XM_001703345)	F R M R L L G A E V R P V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S A A G P H P Y P M M V R F Q S	262		
<i>Bacillus mycoides</i> (AJH19322)	F R M E L L G A K V E S V A A G S G T I K D A W N E A I R Y W V S H V D T T H Y I L G S V L G P H P F P G T V R D F Q S	210		
<i>Thermus thermophilus</i> (YP_144361)	F R M K L L G A E V R P V A A G S R T I K D A T N E A I R D W I T N V R T T E Y I L G S V L G P H P Y P M M V R D F Q S	228		
<i>Pseudomonas amygdali</i> (KKY50726)	F R M K L L G A E I V P V T S G T G T I K D A M N E A I R D W V T N V D S T F Y I L G T V A G P H P Y P A M V R D F Q A	214		
<i>Pseudomonas fluorescens</i> (AJZ93490)	F R M K L L G A E V R P V A G T G T I K D A M N E A I R D W V T N V D S T F Y I G T V A G P H P Y P A M V R D F Q A	216		
<i>Bradyrhizobium elkanii</i> (WP_016847382)	F R M E M L G A K V V P V S G A R T I K D A M N D A I R D W V T N V H D T E Y C I G T V A G P H P Y P M M V R D F Q S	217		
<i>Medicago truncatula</i> (AES70297)	F R M R L L G A E V R P V H A G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	226		
<i>Glycine max</i> (XP_003519001)	F R M R L L G A E V R P V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	293		
<i>Camptotheca acuminata</i> (AAB97526)	F R M R L L G A E V R A V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R E F H A	280		
<i>Vitis vinifera</i> (XP_002281484)	F R M R L L G A E V R A V H A G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	272		
<i>Arabidopsis thaliana_lower</i> (M81620)	F R M R L L G A E V R G V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	289		
<i>Brassica napus</i> (XP_013706880)	F R M R L L G A E V R G V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	286		
<i>Arabidopsis thaliana_upper</i> (M23872)	F R M R L L G A E V R G V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R D F H A	284		
<i>Oryza sativa</i> (AB003491)	F R M K L L G A E V R E V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R E F H K	282		
<i>Zea mays</i> (XP_008662907)	F R M R L L G A E V R A V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R E F H K	293		
<i>Sorghum bicolor</i> (AAL73524)	F R M R L L G A E V R A V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M M V R E F H K	290		
<i>Physcomitrella patens</i> (XP_001752340)	F R M R L L G A E V R P V H S G T A T I K D A T S E A I R D W V T N V E T T H Y I L G S V A G P H P Y P M V R D F H A	282		



	320	340	360	
<i>Synechocystis</i> sp. (WP_010874081.1)	V I G K E T R K Q A L E K W G G K P D V L V A C V G G G S N A M G L F H E F - - V D D E V R L I G V E A A G F G L D T	282		
<i>Microcystis aeruginosa</i> (NC_010296)	V I G E E T R Q Q S L E K W G G L P D I L A C V G G G S N A M G L F H E F - - V K E A S V R L I G V E A A G E S I L A S	285		
<i>Thermosynechococcus elongatus</i> (NP_683264.1)	V I G A E T R Q Q C L E K W G G L P D V L A C V G G G S N A M G L F H E F - - V E E P Q V R L I G V E A A G G G L O T	281		
<i>Gloeobacter violaceus</i> (NP_925704.1)	V I G R E T R Y Q C L E R L G R L P D V L A C V G G G S N A M G L F H D F - - L D E R A V R L V G V E A A G G E T	282		
<i>Ostreococcus tauri</i> (NC_014432)	C I G Q E T R A Q A M E K W G G L P D I L A C V G G G S N A M G L F H E F - - I N D E S V R L I G V E A G G E L A P	305		
<i>Bathycoccus prasinos</i> (NC_024005)	M I G K E V R E Q A M E K W G G K P D I L A C V G G G S N A M G L F H E F - - V N D E S V R L I G V E A G G E G L I	345		
<i>Chlamydomonas reinhardtii</i> (XM_001703345)	V I G R E T R K V Q A Q E K W G G L P D I V A C V G G G S N A M G L F N E F - - I N D T S V R L I G V E A G G E G M N T	320		
<i>Bacillus mycoides</i> (AJH19322)	V I G K E T K K Q Y E A L E G K L P E A V A C I G G G S N A M G M F Y P F - - V H D E E V A L Y G V E A A G K G V H T	268		
<i>Thermus thermophilus</i> (YP_144361)	V I G E E V K R Q S L E K L F R L P D A I A A V G G G S N A M G L F A P F A Y L P E G R P K L I G V E A A G E G L S T	288		
<i>Pseudomonas amygdali</i> (KKY50726)	V I G K E T K E Q M Q E K E G R L P D S I A C V G G G S N A M G L F H P F - - L D D A S V E I I G V E A G G H V O T	272		
<i>Pseudomonas fluorescens</i> (AJZ93490)	V I G K E T R D Q L Q A Q E G R L P D S I A C I G G G S N A M G L F H P F - - L D D Q S V E I I G V E A A G H G E T	274		
<i>Bradyrhizobium elkanii</i> (WP_016847382)	V I G H E T R K Q M Q E A E C R L P D S I A C I G G G S N A M G L F H P F - - L D D P L E I F G V E A A G H G - T	274		
<i>Medicago truncatula</i> (AES70297)	V I G K E T R K Q A L E K W G G K P D I L A C V G G G S N A M G L F H E F - - V D D K D V R L I G V E A S G G E S	284		
<i>Glycine max</i> (XP_003519001)	V I G K E T R K Q A L E K W G G K P D I L A C V G G G S N A M G L F N E F - - V D D K D V R L I G V E A A G F G L D S	351		
<i>Camptotheca acuminata</i> (AAB97526)	V I G K E T R K Q A L E K W G G K P D V L A C V G G G S N A M G L F H E F - - V D D K D V R M I G V E A A G F G L D S	338		
<i>Vitis vinifera</i> (XP_002281484)	V I G K E T R K Q A M E K W G G K P D V L A C V G G G S N A M G L F H E F - - V D D E D V R L I G V E A A G F G L D S	330		
<i>Arabidopsis thaliana_lower</i> (M81620)	V I G K E T R K Q A M E K W G G K P D V L A C V G G G S N A M G L F H E F - - V D D T E V R M I G V E A A G F G L D S	347		
<i>Brassica napus</i> (XP_013706880)	V I G K E T R R Q A M E K W G G K P D V L A C V G G G S N A M G L F H E F - - V D D T E V R M I G V E A A G F G L D S	344		
<i>Arabidopsis thaliana_upper</i> (M23872)	V I G K E T R K Q A L E K W G G K P D V L A C V G G G S N A M G L F H E F - - V D D T E V R M I G V E A A G F G L D S	342		
<i>Oryza sativa</i> (AB003491)	V I G K E T R R Q A L E K W G G K P D V L A C V G G G S N A M G L F H E F - - V D D Q E V R M I G V E A A G F G L D S	340		
<i>Zea mays</i> (XP_008662907)	V I G K E T R R Q A M D K W G G K P D V L A C V G G G S N A M G L F H E F - - V E D Q D V R L I G V E A A G H G V O T	351		
<i>Sorghum bicolor</i> (AAL73524)	V I G K E T R R Q A M D K W G G K P D V L A C V G G G S N A M G L F H E F - - V E D Q D V R L I G V E A A G H G V O T	348		
<i>Physcomitrella patens</i> (XP_001752340)	M I G T E V R R Q A M E K W G G K P D V L A C V G G G S N A M G L F H E F - - E D E D V R L I G V E A A G F G L D T	340		



	380	400	420				
Synechocystis sp. (WP_010874081.1)	G-KHAAATLTM	GKPGVLHG--	AMSYLLQDDKE	GOVTEAHSIS	AGLDYPGVGP	EHSYFLKDIGR	339
Microcystis aeruginosa (NC_010296)	G-KHAAATLTC	GQPGVLHG--	AMSYLLQDSE	GOVTEAHSIS	AGLDYPGVGP	EHSYFLKDSGR	342
Thermosynechococcus elongatus (NP_683264.1)	G-HHAAATLTK	GEGVVLHG--	AMSYLLQDAD	GOVTEAHSIS	AGLDYPGVGP	EHSYFLKDIGR	338
Gloeobacter violaceus (NP_925704.1)	G-KHAAATLTA	GKPGVLHG--	AMSYVLODFQ	GOVTEAHSIS	AGLDYPGVGP	EHSYFLKDIGR	339
Ostreococcus tauri (NC_014432)	GQKHAAATLIT	GTPGVVLHG--	SFSYLIQDEE	GOVTEPHSIS	AGLDYPGVGP	EHAFLKDFGR	363
Bathycoccus prasinos (NC_024005)	GQKHAAATLIT	GKPGVLHG--	SFSYLIQDEE	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDFGR	403
Chlamydomonas reinhardtii (XM_001703345)	T-KHAAATLTM	GTPGVVLHG--	SYSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDVKR	377
Bacillus mycoides (AJH19322)	-KHAATLTK	GSGVVLHG--	SMYLLQND	GOVTEAHSIS	AGLDYPGVGP	EHSYFLKDIGR	325
Thermus thermophilus (YP_144361)	G-RHAASLIGA	GKRGVLHG--	SYMVLLYDHD	GOVTEPHSIS	AGLDYPGVGP	EHSYFLADAGV	345
Pseudomonas amygdali (KKY50726)	E-KHAASLNG	GVPGVVLHGNR	--TYLLQDDG	GOVTEAHSIS	AGLDYPGVGP	EHAFLHEVYKR	329
Pseudomonas fluorescens (AJZ93490)	G-KHAASLNG	GVPGVVLHGNR	--TYLLQDDG	GOVTEAHSIS	AGLDYPGVGP	EHAFLHEVYKR	331
Bradyrhizobium elkanii (WP_016847382)	G-LHAASLAG	GRPGVLHGNR	--TYLLQDDG	GOVTEAHSIS	AGLDYPGVGP	EHAFLHEVYKR	331
Medicago truncatula (AES70297)	G-KHAAATLTK	GEGVVLHG--	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	341
Glycine max (XP_003519001)	G-KHAAATLTK	GEGVVLHG--	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	408
Camptotheca acuminata (AAB97526)	G-KHAAATLTK	GEGVVLHG--	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	395
Vitis vinifera (XP_002281484)	G-KHAAATLTK	GEGVVLHG--	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	387
Arabidopsis thaliana_lower (M81620)	G-KHAAATLTK	GEGVVLHG--	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	404
Brassica napus (XP_013706880)	G-KHAAATLTK	GEGVVLHG--	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDMGR	401
Arabidopsis thaliana_upper (M23872)	G-KHAAATLTK	GEGVVLHG--	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDMGR	399
Oryza sativa (AB003491)	G-KHAAATLTK	GEGVVLHGS	AMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDMGR	399
Zea mays (XP_008662907)	E-KHAAATLTK	GQVGVVLHG--	SMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	408
Sorghum bicolor (AAL73524)	E-KHAAATLTK	GEGVVLHG--	SMSYLLQDDD	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	405
Physcomitrella patens (XP_001752340)	S-KHAAATLTM	GEGVVLHG--	AMSYLLQDDG	GOVTEPHSIS	AGLDYPGVGP	EHSFLKDIGR	397

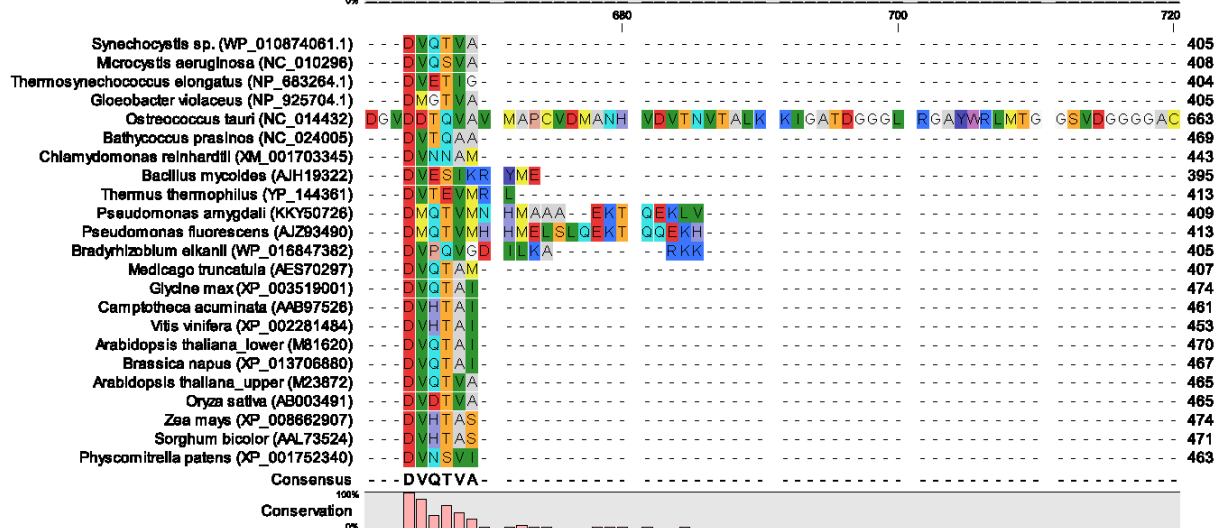
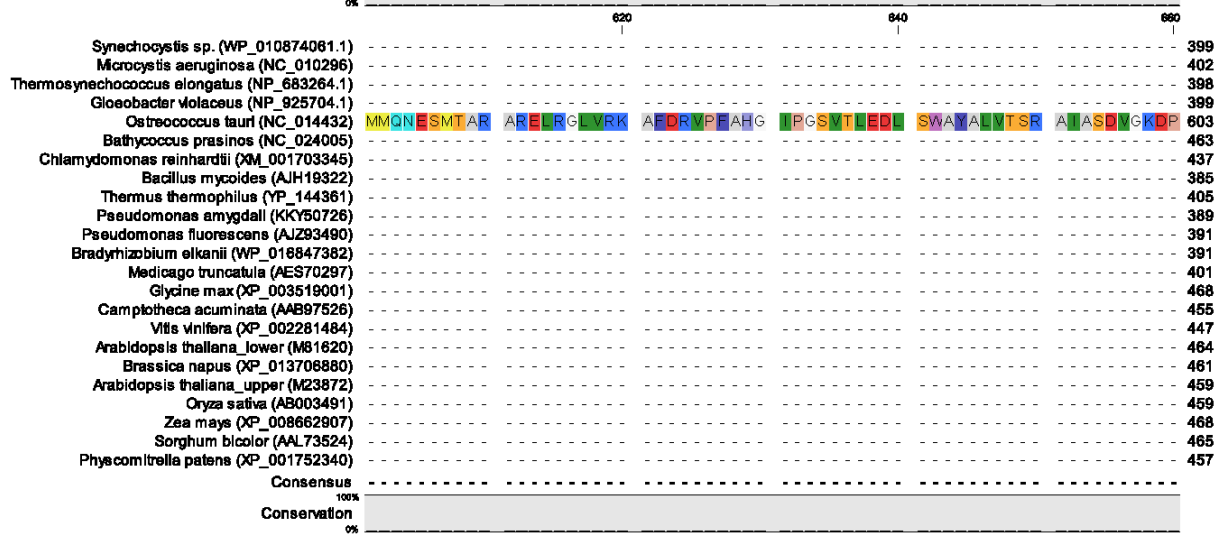
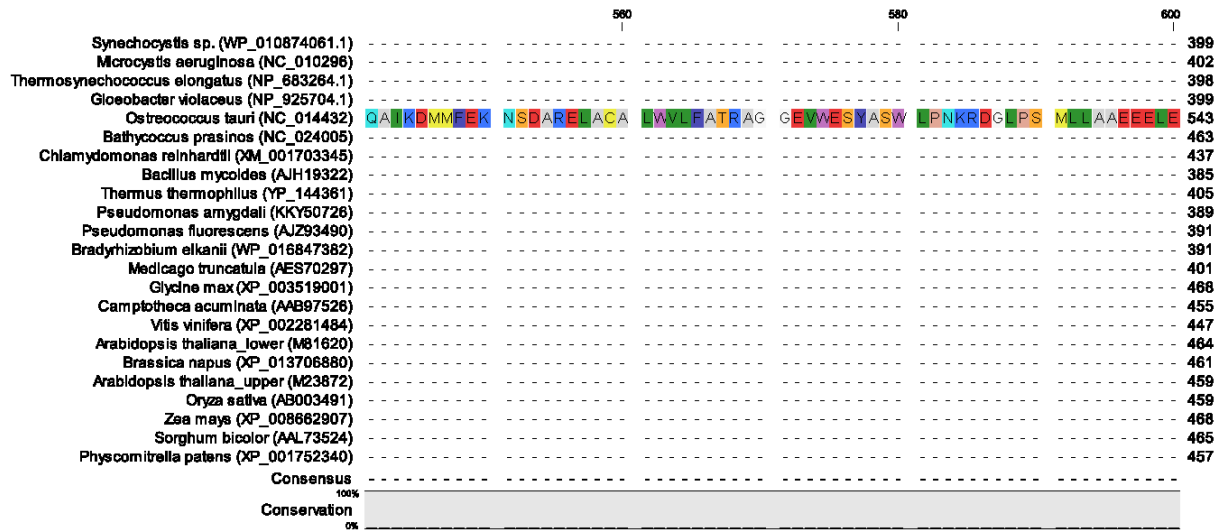


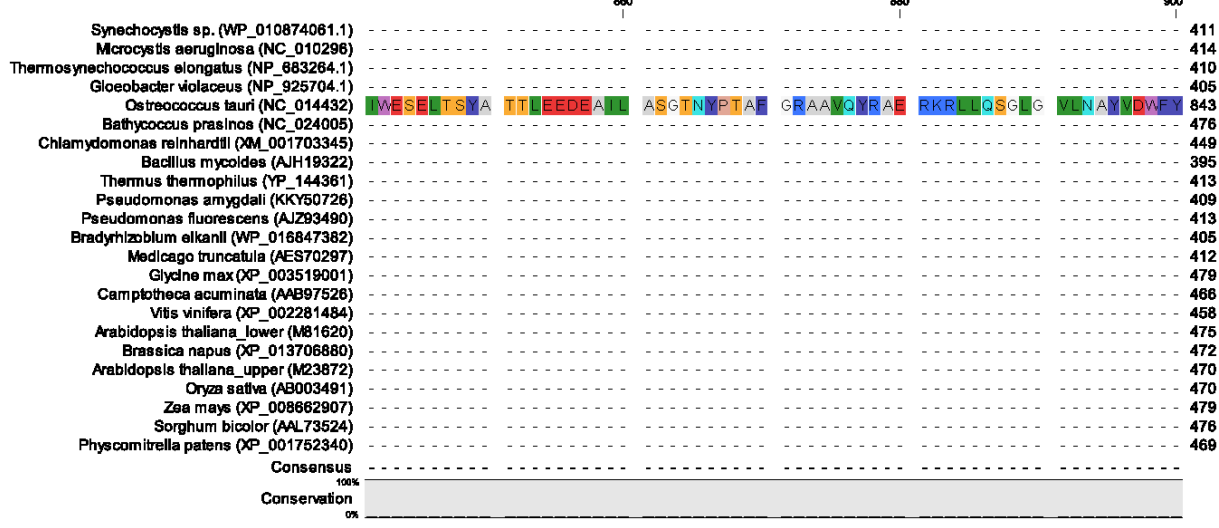
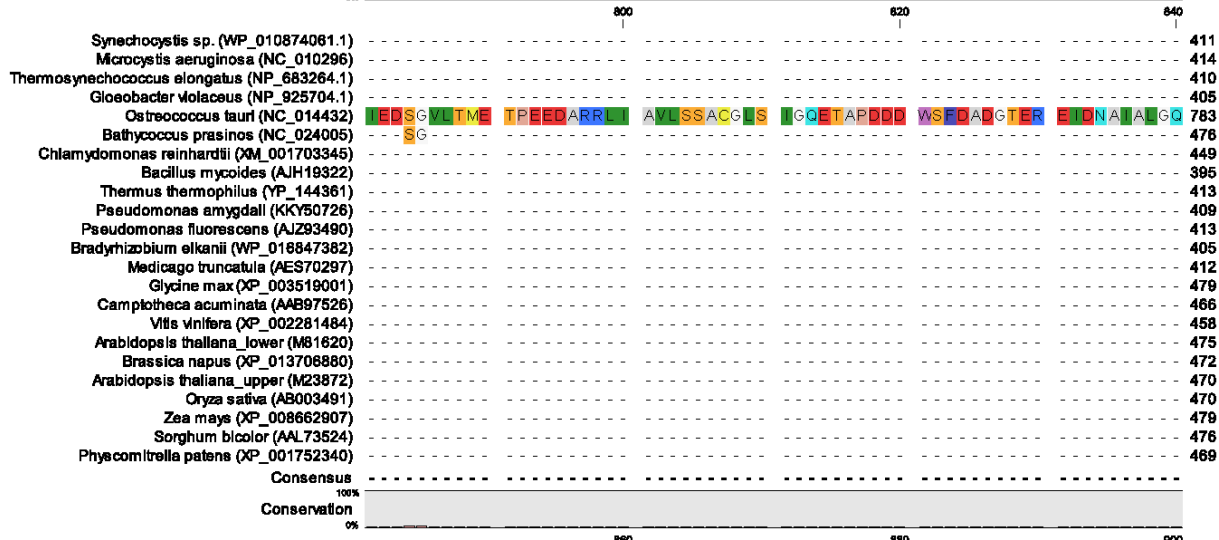
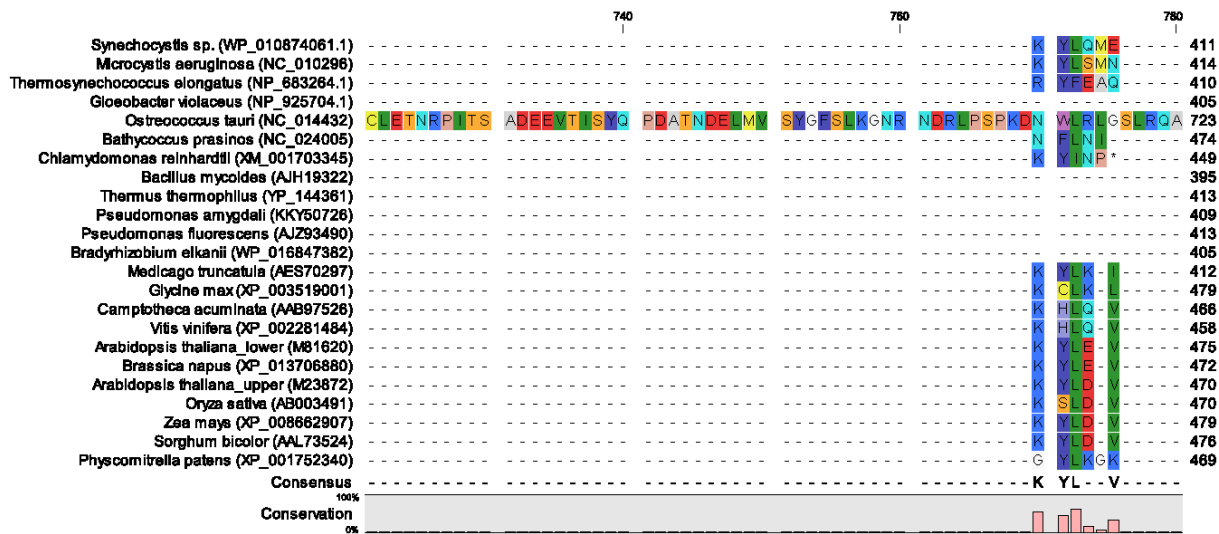
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Synechocystis sp. (WP_010874081.1)	AEYYSVTDEE	AITALQRLSQ	LEGIPALET	AHAFAYLET	CPQLKNGERL	VINCNSGRGDK	399
Microcystis aeruginosa (NC_010296)	AEYYSVTDEE	ALEAFQRVSR	LEGIPALET	AHALAYLET	CPQVITGSPR	VINCNSGRGDK	402
Thermosynechococcus elongatus (NP_683264.1)	AEYYSVTDEE	AVAACVRLAQ	LEGIPALET	AHALAYLET	CPQLTGSPPR	VINCNSGRGDK	398
Gloeobacter violaceus (NP_925704.1)	AEYYSVTDEE	ALAAALSLVCS	TEGLIPALET	AHALAYLET	ASQLHSQELV	VINCNSGRGDK	399
Ostreococcus tauri (NC_014432)	EYHAIIDKE	ADAFVNTSR	EGIPALET	SHALAYLEK	CPGLPNTKV	VINCNSGRGDK	423
Bathycoccus prasinos (NC_024005)	AEYYPATDQE	ALDAFVFTSR	LEGIPALET	SHALAYLEK	CPTLPDKCKV	VINCNSGRGDK	463
Chlamydomonas reinhardtii (XM_001703345)	AEYYSATDDE	ALEGFQLLSK	LEGIPALET	SHALAYLEK	TPTLPSGTRV	VINCNSGRGDK	437
Bacillus mycoides (AJH19322)	SYHSITDEE	ALEAFQLLAK	KEGIPALEE	SHAVAYALK	APQMKKDEGL	VINCNSGRGDK	385
Thermus thermophilus (YP_144361)	AEYASVTDEE	ALEGFKLLAR	EGIPALEE	AHAAIAAAKV	WPEMDKDDVV	VINCNSGRGDK	405
Pseudomonas amygdali (KKY50726)	AEYYSITDDE	ALDAFHQCC	EGIPALET	AHALAEAMKR	ATNLRDDHLM	VINCNSGRGDK	389
Pseudomonas fluorescens (AJZ93490)	QYYSITDDE	ALDAFHQCC	EGIPALEE	AHALAEVFKR	APTLPKDHLM	VINCNSGRGDK	391
Bradyrhizobium elkanii (WP_016847382)	WYYSATDEE	ALAAAFQLLSR	LEGIPALEE	AHALAYLEK	APQRPKDHLM	VINCNSGRGDK	391
Medicago truncatula (AES70297)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	401
Glycine max (XP_003519001)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	468
Camptotheca acuminata (AAB97526)	AEYYSCTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	455
Vitis vinifera (XP_002281484)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	447
Arabidopsis thaliana_lower (M81620)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	464
Brassica napus (XP_013706880)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	461
Arabidopsis thaliana_upper (M23872)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	459
Oryza sativa (AB003491)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	459
Zea mays (XP_008662907)	AEYDSITDQE	ALDAFKRVSR	EGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	468
Sorghum bicolor (AAL73524)	AEYYSVTDEE	ALEAFKRVSR	LEGIPALEE	SHALAYLEK	CPTLPNGTKV	VINCNSGRGDK	465
Physcomitrella patens (XP_001752340)	AEYYSVTDEE	ALEAFQRVSR	LEGIPALEE	SHALAYLET	CPTLPNGTKV	VINCNSGRGDK	457

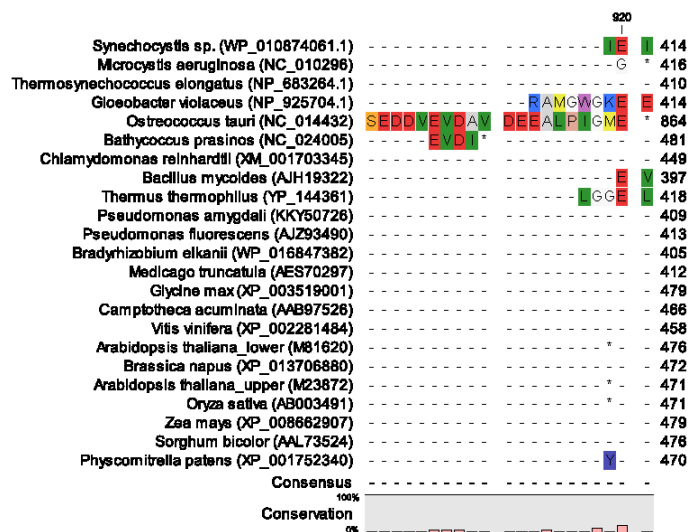


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Synechocystis sp. (WP_010874081.1)	-----	-----	-----	-----	399		
Microcystis aeruginosa (NC_010296)	-----	-----	-----	-----	402		
Thermosynechococcus elongatus (NP_683264.1)	-----	-----	-----	-----	398		
Gloeobacter violaceus (NP_925704.1)	-----	-----	-----	-----	399		
Ostreococcus tauri (NC_014432)	XXXXXXXXTTS	MYGKMSVELL	LEMPLDVCLY	APVGDGSAAS	ADDTAKAVVA	SWERRNRWEP	483
Bathycoccus prasinos (NC_024005)	-----	-----	-----	-----	463		
Chlamydomonas reinhardtii (XM_001703345)	-----	-----	-----	-----	437		
Bacillus mycoides (AJH19322)	-----	-----	-----	-----	385		
Thermus thermophilus (YP_144361)	-----	-----	-----	-----	405		
Pseudomonas amygdali (KKY50726)	-----	-----	-----	-----	389		
Pseudomonas fluorescens (AJZ93490)	-----	-----	-----	-----	391		
Bradyrhizobium elkanii (WP_016847382)	-----	-----	-----	-----	391		
Medicago truncatula (AES70297)	-----	-----	-----	-----	401		
Glycine max (XP_003519001)	-----	-----	-----	-----	468		
Camptotheca acuminata (AAB97526)	-----	-----	-----	-----	455		
Vitis vinifera (XP_002281484)	-----	-----	-----	-----	447		
Arabidopsis thaliana_lower (M81620)	-----	-----	-----	-----	464		
Brassica napus (XP_013706880)	-----	-----	-----	-----	461		
Arabidopsis thaliana_upper (M23872)	-----	-----	-----	-----	459		
Oryza sativa (AB003491)	-----	-----	-----	-----	459		
Zea mays (XP_008662907)	-----	-----	-----	-----	468		
Sorghum bicolor (AAL73524)	-----	-----	-----	-----	465		
Physcomitrella patens (XP_001752340)	-----	-----	-----	-----	457		

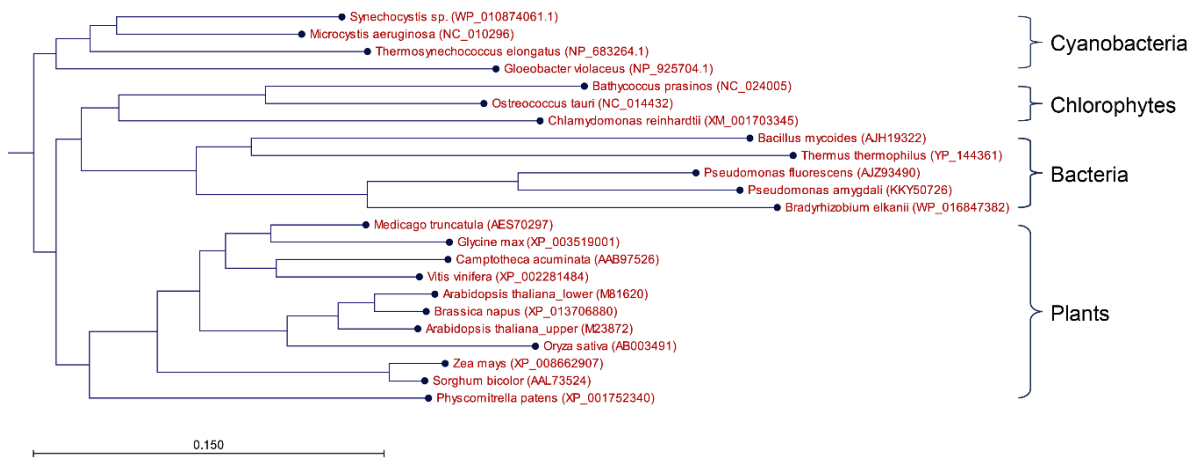




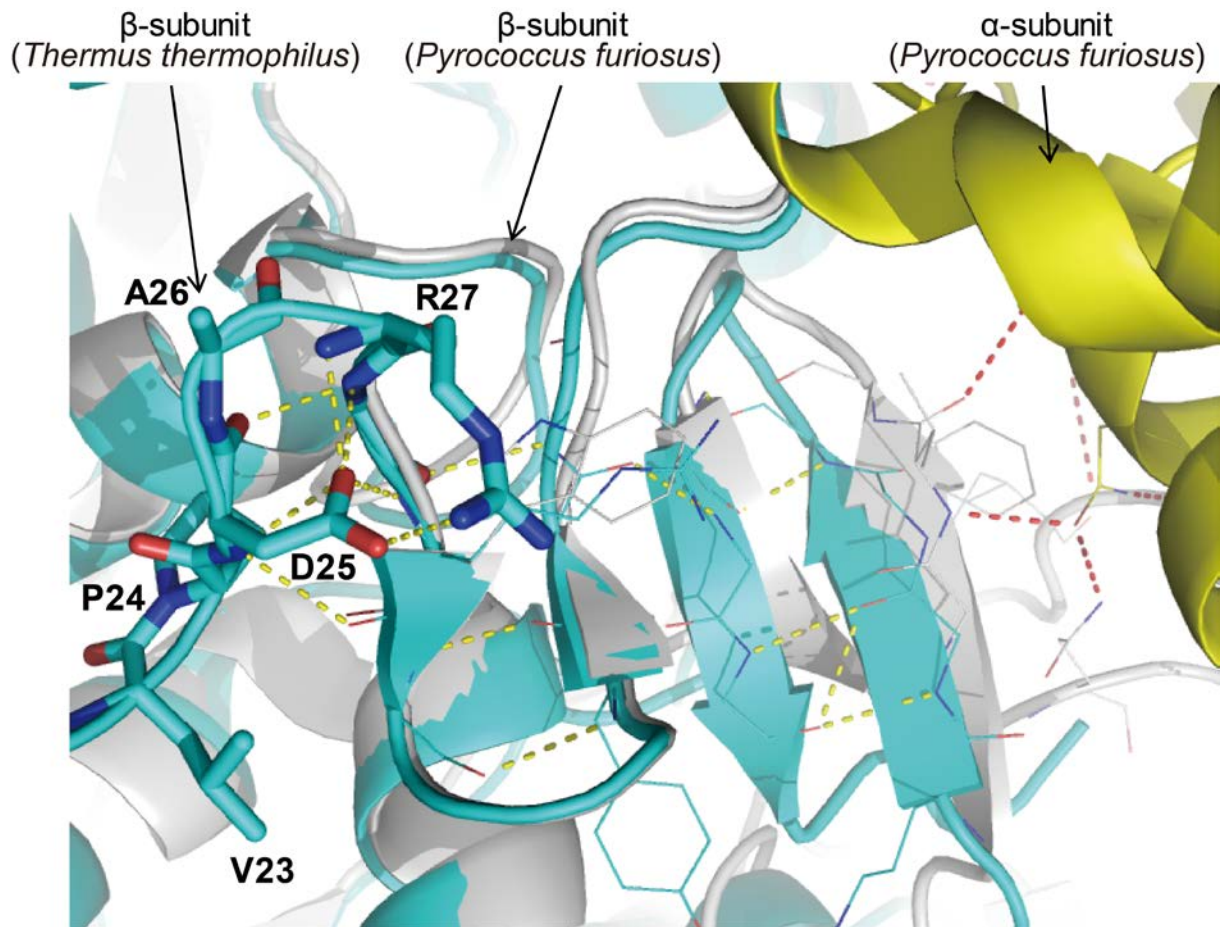




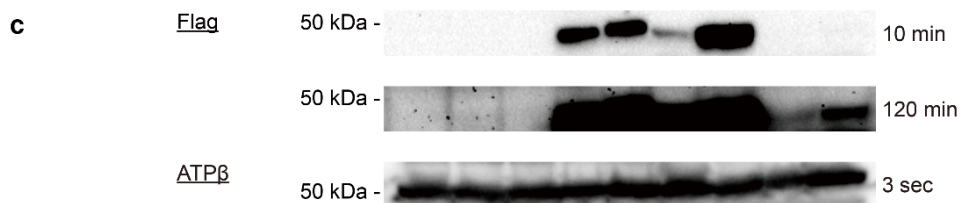
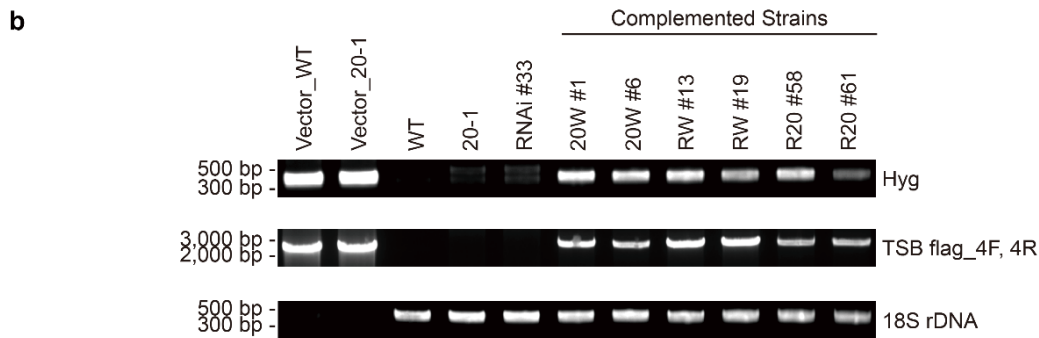
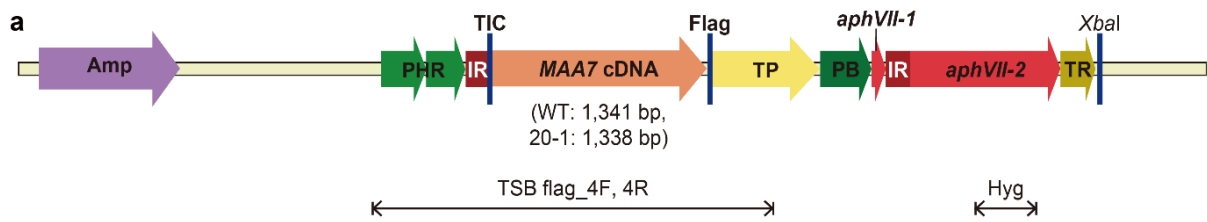
Supplementary Fig. 1. Alignment of the full-length TSB amino acid sequences from organisms representing three kingdoms.



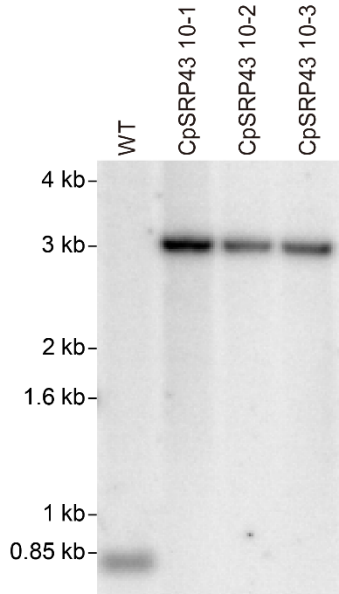
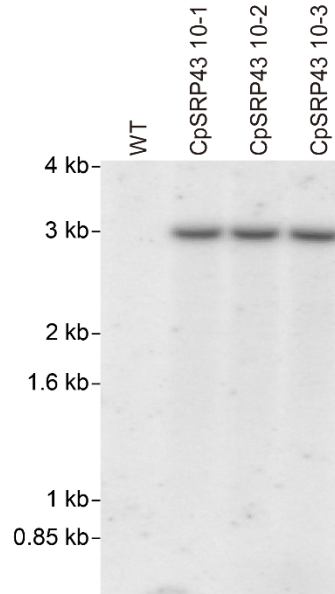
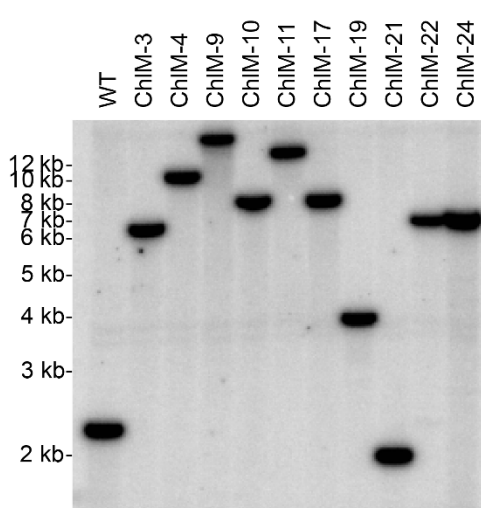
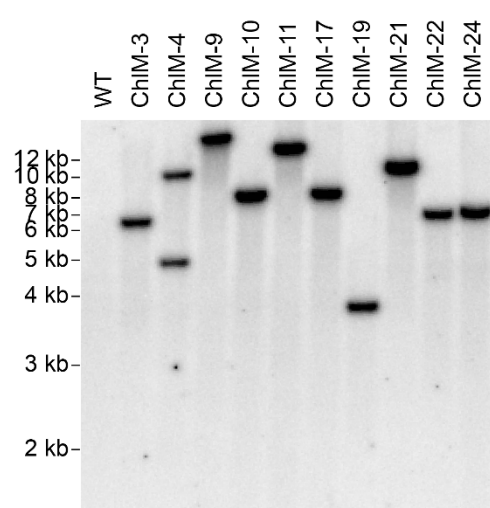
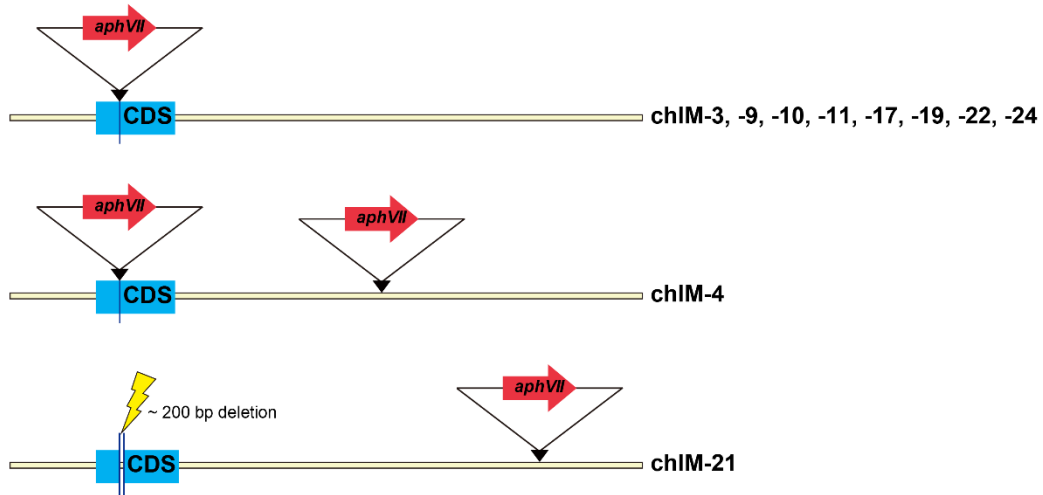
Supplementary Fig. 2. Phylogenetic tree of the TSB amino acid sequences.



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.

a**3' coding region of *CpSRP43*****b*****aphVII* coding region****c****5' coding region of *ChIM*****d*****aphVII* coding region****e**

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)	
MAA7	M2_F	CAACGTTTGCCGTTGGTATG	Amplifying Cas9 cut site of <i>MAA7</i> (<i>in vitro</i>)	783	
	M2_R	TTCAGGATAGCCTCCATCTCC			
	I202TSB_3F	GTCGCCGTTTCCATTGCAGGATGGCCATG GAAGCTTGATAACAATGAACATCACTGCCCC TACTC		Vector construction for complementation of <i>MAA7</i>	1,455
		I202TSB_3R	TGATACAGGCGGTCCAATCTGAATTGGGCT GCAGGAATTCGATTCACTTGTCGTCGTCGT CCTTGTAGTCAGGGTTGATGTACTTCATGGC GTT		
	aph 72_L		CCCCATTCCGAGGTCTTCC	Confirmation of complemented <i>MAA7</i> strains (Hygromycin resistant)	387
	aph 72_R	AAGGTGAAGGCGAGCAGTTC			
	TSB flag_4F	GCGCAATTAACCCTCACT	Confirmation of complemented <i>MAA7</i> strains (transgenic <i>MAA7</i> gene)	2,477 for WT	
	TSB flag_4R	CAAACCTCACATGGCCT		2,474 for <i>MAA7</i> 20-1	
	SR6	GTCAGAGGTGAAATCTTGG	Confirmation of complemented <i>MAA7</i> strains (18S rDNA)	380	
	SR9	AACTAAGAACGGCATGCAC			
CpSRP43	SRP43_2F	TATTAGGGCATCGACAGG	Amplifying Cas9 cut site of <i>CpSRP43</i> (<i>in vitro</i>)	795	
	SRP43_2R	TGGAAGAATTGGAATGGG			
	SRP43_1F	CAAATACCATCGGCGTGTGG	Region A	509	
	PRR2	ACTGACCCACAGCTGTCCTAAGCC			
	PRR2_RC	GGCTTAGGACAGCTGTGGGTCAGT	Region B	1,662	
	hpt1579_R	TACCGCTTCAGCACTTGAGA			
	aph72_L	CCCCATTCCGAGGTCTTCC	Region C	1,089	
	SRP43_1R	GCGACACCTCGTGAAGCATA			
	aph7_1471F	TGGTGCAACTGCATCTCAAC	Southern blot of <i>aphVII</i> probe	203	
	aph7_1114R	CCAGAGGAAGTGCGCCAGTTCCT			
SRP43_4F	AAATGGAAGGTGAGAGCTGG	Southern blot of <i>CpSRP43</i> probe	305		
SRP43_4R	GTCAGATCCGTCCTGTGGAA				
ChIM	CrChIM_F1	GTGTTGCAGAGGTGTCTCAGTTGCCAACA	Amplifying Cas9 cut site of <i>ChIM</i> (<i>in vitro</i>)	1,010	
	CrChIM_R3	CCGTCTCGCCGTAGATCTTGCGCCA			
	aph7_1471F		Hygromycin resistant	203	
	aph7_1114R				
	CrChIM_F	TCGCTGCGTGGCGGAAGCACTA	Region D in ChIM-3, 22, 24	2,000 for ChIM-3	
	aph7_1471F			1,700 for ChIM-22	
	CrChIM-F1		Region D in ChIM-9	500	
	psaD_ter_R	GGTCTTCTCAATGGGTGTGGCACGT			
	CrChIM_F1		Region D in ChIM-10, 17	500	
	aph7_1471F				
	CrChIM_F3	GCATGGCAAGCTGGCGTCAGCA	Region D in ChIM-19	650	
	aph7_R2	TTCCGGTCCGGTTCGTGCCGTCCAT			
	psaD_ter_R1	CCTGTGGCTAATTGACCGTG	Region E in ChIM-3, 22, 24	400 for ChIM-3	
	CrChIM_R1	GCACTCCTGCACCCCTTCTCACCGCT		1,000 for ChIM-22, 24	
	aph7_1471F		Region E in ChIM-9, 19	700 for ChIM-9	
	CrChIM_R1			500 for ChIM-19	
	CrChIM_F1		Southern blot of <i>ChIM</i> probe	257	
	CrChIM_R2	CCGAGGTAAAAGGCTTGGTGTGGTTAGGCA			
ChIM_RT_F	ATGCAGTGCCTAACACCAAGCCT	RT-PCR (<i>ChIM</i>)	361		
ChIM_RT_R	CCGTCTCGCCGTAGATCTTGCGCCA				
IDA5_F	ATCGTGCGCGACATCAAGGA	RT-PCR (loading control)	464		
IDA5_R	TACTCGGACTTGGCGATCCA				