

ONLINE RESOURCES

Genetic and genomic analysis of RNases in model cyanobacteria

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Online Resource 1 Plasmids used in this study

Plasmid ID	Description/Purpose	Resistance marker	Reference
pJCC248	pUC19- $\Delta A0788$:Km ^R	Amp/Km	This work
pJCC249	pUC19- $\Delta A0061$:Sp ^R	Amp/Sp	This work
pJCC250	pUC19- $\Delta A2542$:Km ^R	Amp/Km	This work
pJCC251	pUC19- $\Delta A0384$:Gm ^R	Amp/Gm	This work
pJCC252	pUC19- $\Delta A1273$:Km ^R	Amp/Km	This work
pJCC253	pUC19- $\Delta A0574$:Sp ^R	Amp/Sp	This work
pJCC254	pUC19- $\Delta A1543$:Sp ^R	Amp/Sp	This work
pJCC255	pUC19- $\Delta A1066$:Gm ^R	Amp/Gm	This work
pJCC256	pUC19- $\Delta glpK$:Amp ^R - <i>A0061</i>	Amp	This work
pJCC257	pUC19- $\Delta glpK$:Km ^R - <i>A1543</i>	Km	This work
pUC19	Cloning vector	Amp	Invitrogen
pSRA81	Source of Sp ^R	Sp	(Frigaard et al. 2004)
pBBR1MCS5	Source of Gm ^R	Gm	(Kovach et al. 1995)
pET28b(+)	Source of Km ^R	Km	Novagen

Frigaard NU, Maresca JA, Yunker CE, Jones AD, Bryant DA (2004) Genetic manipulation of carotenoid biosynthesis in the green sulfur bacterium *Chlorobium tepidum*. *J Bacteriol* 186 (16):5210-5220

Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, 2nd, Peterson KM (1995) Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* 166 (1):175-176

Online Resource 2 Oligonucleotides used in this study for generation of plasmids and segregation analysis

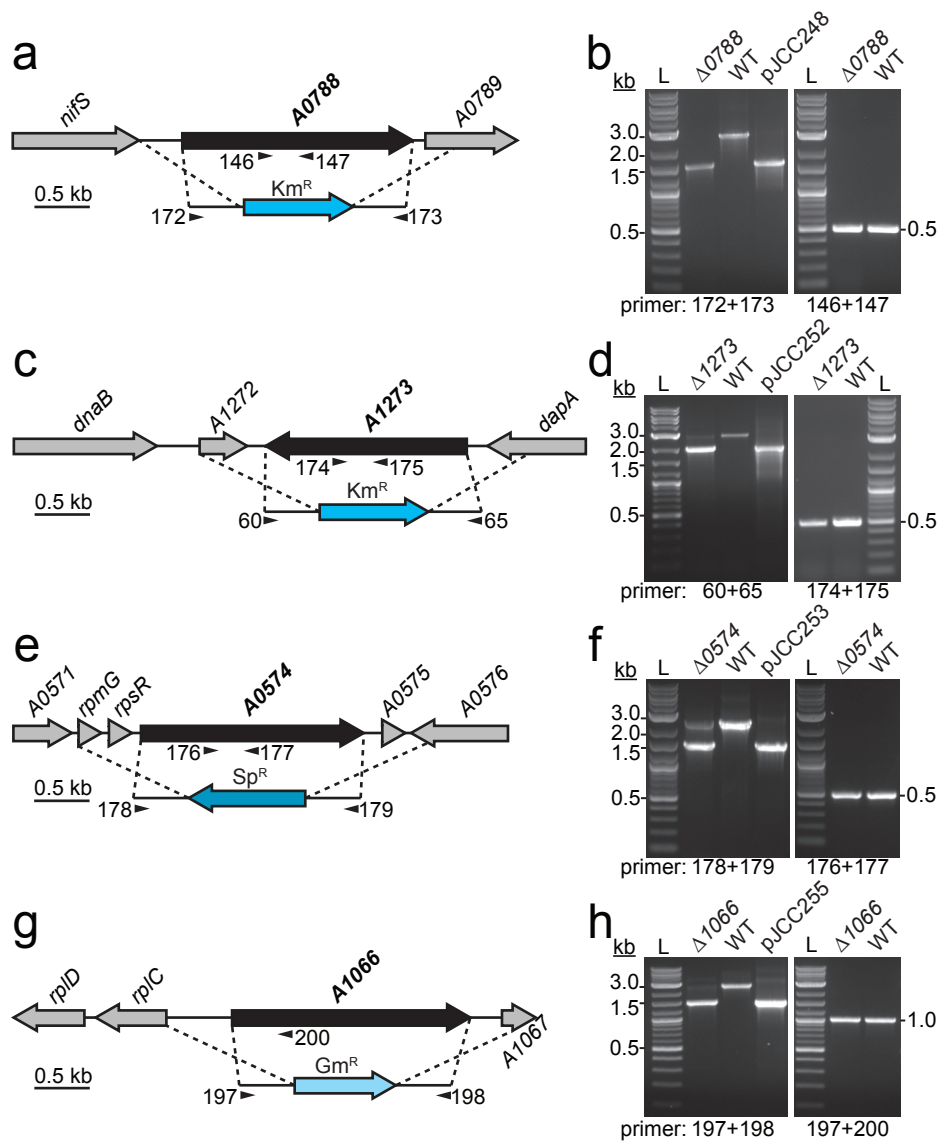
Oligo ID	Sequence (5'-3')	Description	Purpose	Reference
ΔA0788 strain				
JC128	gctcggtagccggggatcctCGATATCGATCACGTCGC	A0788_upstream_F	KO/Segregation	This work
JC129	tgagcgtcagTCAAATTCCTCGATTTTTTTC	A0788_upstream_R	KO	This work
JC130	aggaattgaCTGACGCTCAGTGGAAACG	KmR_F	KO	This work
JC131	taattgggtTTCAGGTGGCACTTTTCG	KmR_R	KO	This work
JC132	gccacctgaaAACCCAATTACCGTTAACCTAAGG	A0788_downstream_F	KO	This work
JC133	tgcatgctgcaggtcgactCGGACTTCAGCGACGGTG	A0788_downstream_R	KO/Segregation	This work
JC146	ACATGGATTCTCGCCATGAT	A0788_gene_specific_F	Segregation	This work
JC147	CGGCTTACTCGAAACCTCTG	A0788_gene_specific_R	Segregation	This work
JC172	TCATCTCACCCAGCCATACA	A0788_flanking_F	Segregation	This work
JC173	GGGCTTGTAACAGACTTGC	A0788_flanking_R	Segregation	This work
ΔA0061 strain				
JC42	gctcggtagccggggatcctTCTTGCCCCATAAGCAG	A0061_upstream_F	KO/Segregation	This work
JC43	aattcactggAAAAGCGCCGGAGATCT	A0061_upstream_R	KO	This work
JC44	gggctctttCCAGTGAATTCGAGCTCGG	SpR_F	KO	This work
JC45	tagtattccCAAGCTTGCATCCCTGCA	SpR_R	KO	This work
JC46	tgcaagcttgGAAATACTAAGATAGCAAAAGTAGGGAACCTTTCG	A0061_downstream_F	KO	This work
JC47	tgcatgctgcaggtcgactGCAGCGGCAATCCACGTC	A0061_downstream_R	KO/Segregation	This work
JC140	TCCCCGACAAAAATTATGA	A0061_gene_specific_F	Segregation	This work
JC141	AGCAAAGTATCCCCAGCTT	A0061_gene_specific_R	Segregation	This work
ΔA2542 strain				
JC48	gctcggtagccggggatcctATGATCACGGGATATATTG	A2542_upstream_F	KO/Segregation	This work
JC49	agaccctgtaGGTTAAGCGAAACTTATAATG	A2542_upstream_R	KO	This work
JC50	tcgcttaaccTACGGGGTCTGACGCTCA	KmR_F	KO	This work
JC51	tctgcccgaGATTTTGCCGATTTTCGGC	KmR_R	KO	This work
JC52	cggaacaatcTTGGGCGAGAAAATGGCT	A2542_downstream_F	KO	This work
JC53	tgcatgctgcaggtcgactAAAGCAATCTCGTTATCAAAAG	A2542_downstream_R	KO/Segregation	This work
JC142	GCGATCGCTCTTCTGTATC	A2542_gene_specific_F	Segregation	This work
JC143	GAATTTCACCGTGGTTTGTCT	A2542_gene_specific_R	Segregation	This work
ΔA0384 strain				
JC134	gctcggtagccggggatcctCAGCAAAAAATCTGTAGTTTG	A0384_upstream_F	KO/Segregation	This work
JC135	tatgcatgegTGGTTTTCTCGTCCCTAT	A0384_upstream_R	KO	This work
JC136	gagaaaaaccaCGCATGCATAAAAACTGTTG	GmR_F	KO	This work
JC137	gtcgatcataTTAGGTGGCGGTACTTGG	GmR_R	KO	This work
JC138	cgccacactaaTATGATCGACTCTTTTGTGTTTC	A0384_downstream_F	KO	This work
JC139	tgcatgctgcaggtcgactTGGGGCGTCTTTTATTCG	A0384_downstream_R	KO/Segregation	This work
JC144	GTCCTGACATACCGCTGA	A0384_gene_specific_F	Segregation	This work
JC145	CAGACCGTCTTGGGGTAAA	A0384_gene_specific_R	Segregation	This work
ΔA1273 strain				
JC60	gctcggtagccggggatcctGCGCTGGCCATTAACAG	A1273_upstream_F	KO/Segregation	This work
JC61	tgagcgtcagTGTAGGAAAAGGATTTGGGATC	A1273_upstream_R	KO	This work
JC62	ttttctacaCTGACGCTCAGTGGAAACG	KmR_F	KO	This work
JC63	aaggagttttTTCAGGTGGCACTTTTCG	KmR_R	KO	This work
JC64	gccacctgaaAAAACCTCCTTAGATTTGCTTG	A1273_downstream_F	KO	This work
JC65	tgcatgctgcaggtcgactGATGATAGTCTAACTTACCTTTGATG	A1273_downstream_R	KO/Segregation	This work
JC174	GCGATCATCAGACGATCAAT	A1273_gene_specific_F	Segregation	This work
JC175	GGCGAAAAAGGTGTTCTCTG	A1273_gene_specific_R	Segregation	This work
ΔA0574 strain				
JC148	gctcggtagccggggatcctCCGAATGTCGCCAATA	A0574_upstream_F	KO	This work
JC149	aattcactggCAGCTTTGTGCTTAACG	A0574_upstream_R	KO	This work
JC150	aacaagctgCCAGTGAATTCGAGCTCGG	SpR_F	KO	This work
JC151	gcttttgatcCAAGCTTGCATGCCTGCA	SpR_R	KO	This work
JC152	tgcaagcttgGATCAAAAAGCCGACAAAAC	A0574_downstream_F	KO	This work
JC153	tgcatgctgcaggtcgactCGGAGCTAACACCGATG	A0574_downstream_R	KO	This work
JC176	TCAAACGCAAAGGGGATTAC	A0574_gene_specific_F	Segregation	This work
JC177	ACTGAGGCCATCGTCAATTT	A0574_gene_specific_R	Segregation	This work
JC178	TGTGCCCTTCATCAACAAA	A0574_flanking_F	Segregation	This work
JC179	TCCAAGTGGAGTTGGGATTC	A0574_flanking_R	Segregation	This work
ΔA1543 strain				
JC188	gctcggtagccggggatcctCCATTGATCCAGGCGAAT	A1543_upstream_F	KO	This work
JC189	aattcactggTACCCGTCTATGACTCAG	A1543_upstream_R	KO	This work
JC190	agacgggtgaCCAGTGAATTCGAGCTCGG	SpR_F	KO	This work
JC191	atagttttgcCAAGCTTGCATGCCTGCA	SpR_R	KO	This work
JC192	tgcaagcttgGCAAAACTATCCTTTTCGTTAGAAAAGTAAAGTGAACGACAAC	A1543_downstream_F	KO	This work
JC193	tgcatgctgcaggtcgactGTGGGGCGATCGCCGGGG	A1543_downstream_R	KO	This work
JC180	CGCGATATTCGTACCAAGTCA	A1543_gene_specific_F	Segregation	This work
JC181	TCAGGAAACCTCGATGFTC	A1543_gene_specific_R	Segregation	This work
JC182	AAAAAGAGGGAAGCATGCAA	A1543_flanking_F	Segregation	This work
JC183	TATGCTGGGTTTGTCTAGCC	A1543_flanking_R	Segregation	This work
ΔA1066 strain				
JC160	gctcggtagccggggatcctGAAAGAGTTGTGTTCTTGTGTC	A1066_upstream_F	KO	This work

JC161	atgcatgcgctATTGCGTGTTCCTTTTG	<i>A1066_upstream_F</i>	KO	This work
JC162	acacgcaataGCGCATGCATAAAAACTGTTGTAATTC	GmR_F	KO	This work
JC163	atgtttatattAAACAGGCCACGCGCCAG	GmR_R	KO	This work
JC164	gggcctgtttAAATATAAAACATCACTATTTTTCGGTG	<i>A1066_downstream_F</i>	KO	This work
JC165	tgcattcctgcaggtcgactGTCAATGATGCCCGCTGA	<i>A1066_downstream_R</i>	KO	This work
JC200	TTCGGCTCTTCGTGGTTTT	<i>A1066_gene_specific_R</i>	Segregation	This work
JC197	TTGGGAGTTCAAGGAACCAG	<i>A1066_flanking_F</i>	Segregation	This work
JC198	CGTTGAAAAGGGGTTTGAGA	<i>A1066_flanking_R</i>	Segregation	This work

Complementation Analysis

JCC207	agctcggtaccgggTGAAGCGATTGGCTATGATC	<i>glpK_upstream_F</i>	for pUC19- Δ <i>glpK_NS</i>	This work
JCC208	ggggatccTTTTTTAAATGGGTAAATTAGGTC	<i>glpK_upstream_BamHI_R</i>	for pUC19- <i>glpK_NS</i>	This work
JCC209	ccatttaaaaaaggatccCCCCCTTGCCTACAGC	<i>glpK_downstream_BamHI_F</i>	for pUC19- <i>glpK_NS</i>	This work
JCC210	caggtcgactctagagGAAACGAGATTATCTAAAACAGAAGC	<i>glpK_upstream_R</i>	for pUC19- <i>glpK_NS</i>	This work
JCC263	athtaaccatttaaaaaagctcgagTACGGGGTCTGACGCTCA	KmR_F	for pUC19- <i>glpK:KmR_NS</i>	This work
JCC264	taggcaagaggggggatccGATTTTGCCGATTCGGC	KmR_F	for pUC19- <i>glpK:KmR_NS</i>	This work
JCC368	athtaaccatttaaaaaaggatccGGAAATGTGCGCGGAACC	AmpR_F	for pUC19- <i>glpK:AmpR_NS</i>	This work
JCC369	taggcaagaggggggatccACGCTCAGTGGAACGAAAAC	AmpR_R	for pUC19- <i>glpK:AmpR_NS</i>	This work
JCC374	aaccatttaaaaaagatcATGGAACCCTAAATCTGG	<i>A0061_terminator_region_F</i>	Complementation	This work
JCC375	cgcgacatttccgatacTTATAGAGAGTAGACGAAAATCAC	<i>A0061_Promoter_region_R</i>	Complementation	This work
JCC282	ttaaccatttaaaaaagctcgaGAACGCAATAGGAAAAGTTAACTCAATTTATTAGTTGAG	<i>A1543_promoter_region_F</i>	Complementation	This work
JCC283	cgtcagacccctacCCTAGGCCACCGGGGGCC	<i>A1543_terminator_region_R</i>	Complementation	This work
JCC397	TCTCGATCCTGCAAGGGG	<i>glpK_flanking_F</i>	Segregation of <i>glpK</i>	This work
JCC398	AGGCTTCATGATCAAGGGAA	<i>glpK_flanking_R</i>	Segregation of <i>glpK</i>	This work
JCC399	CACCGCTGGGGCTTGTACC	<i>glpK_gene_specific_R</i>	Segregation of <i>glpK</i>	This work

Uppercase nucleotides are gene-specific; lowercase indicate homologous flanking regions for Gibson assembly reaction. KO, generation of knock out mutant. NS, neutral site.



Online Resource 3 Segregation analysis of ribonucleases in PCC7002

Targeted gene deletion via homologous recombination was used to replace the entire ORF of *A0788*, *A1273*, *A0574*, and *A1066* with an antibiotic resistance cassette (**a**, **c**, **e**, and **g**). Insertion of the cassette into each of these genes was confirmed by PCR using primer pairs flanking the insert (Left gel panel in **b**, **d**, **f**, and **h**). WT genomic DNA was used as the negative control and the plasmid (pJCC#) used to generate the strain was used as a positive size control. PCR using primers that amplify the WT gene (Right gel panel in **b**, **d**, **f**, and **h**) indicates that these strains are heterozygous at the locus of interest. Arrows and numbers below antibiotic resistance cassette and gene in **a**, **c**, **e**, and **g** indicate approximate regions of flanking and gene specific primers respectively. For the gene specific reaction shown in **g** and **h**, a flanking primer and gene specific primer combination was used to amplify a 1 kb band that should not be present in a fully segregated mutant. Plasmids used as the positive control are listed in Online Resource 1. All primers used in segregation analysis are listed in Online Resource 2.

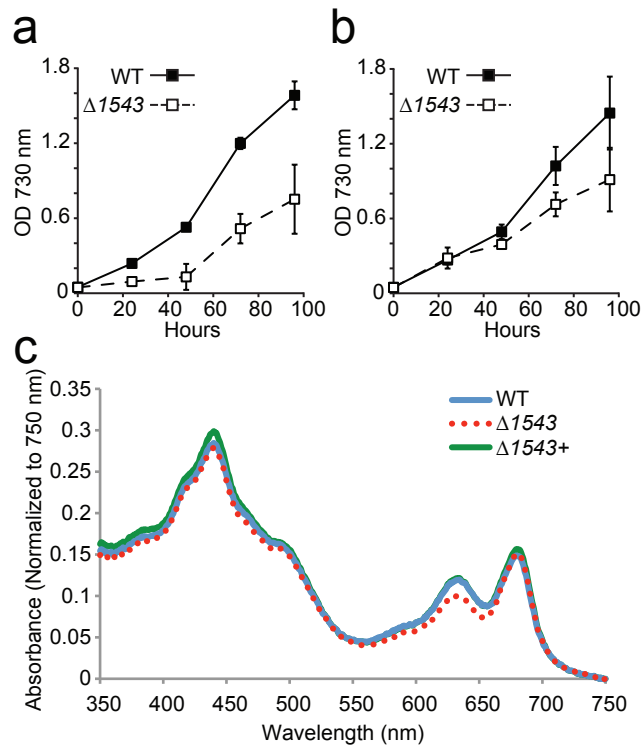
Online Resource 4 Analysis of RNase III-like proteins in diverse cyanobacterial genomes

Genome	Subsection ^a	#RNase III, #MiniIII	RNase III Locus Tag	Mini-III Locus Tag	AA Seq Length	RNase III Signature Motif	Mini-III Signature Motif	#RNase III domains	dsRBD Present	Other Domains
<i>Acaryochloris marina</i> MBIC11017	1	1,1	AM1_1717		247	EQLEFVGDS		1	Y	
<i>Anabaena cylindrica</i> PCC 7122	4	3,1	Anacy_4655	AM1_5197	143	ERLEFLGDA	AALAYIGDA	1	N	
			Anacy_0811		228	ERLEFLGDA		1	Y	
			Anacy_3962		240	EQLEFVGDA		1	Y	
					395	EQLEFLGDC		1	Y	
<i>Anabaena</i> sp. PCC 7108	4	2,1	Ana7108_1646	Anacy_3641	160		SALAYLGDS	1	N	
			Ana7108_0695		230	ERLEFLGDA		1	Y	
					245	EQLEFVGDA		1	Y	
<i>Anabaena</i> sp. PCC 7120	4	2,1	all4107	Ana7108_2529	193		SALAYLGDA	1	N	
			alr0280		202	EQLEFVGDA		1	Y	
					228	ERLEFLGDA		1	Y	
<i>Anabaena variabilis</i> ATCC 29413	4	2,1	Ava_3081	alr1158	161		AALAYVGDA	1	N	
			Ava_0797		228	ERLEFLGDA		1	Y	
					242	EQLEFVGDA		1	Y	
<i>Arthrospira platensis</i> NIES-39	3	2,0	NIES39_Q02990	Ava_4412	161		AALAYVGDA	1	N	
			NIES39_N00330		246	EQLEFVGDS		1	Y	
<i>Calothrix</i> sp. PCC 6303	4	2,1	Cal6303_1320		395	ELLEFLGDS		1	Y	
			Cal6303_1680		228	ERLEFLGDA		1	Y	
					239	EQLEFVGDA		1	Y	
<i>Calothrix</i> sp. PCC 7103	4	2,1	Cal7103DRAFT_00026520	Cal6303_1532	160		KSLAYLGDA	1	N	
			Cal7103DRAFT_00079520		240	EQLEFVGDA		1	Y	
<i>Calothrix</i> sp. PCC 7507	4	2,1	Cal7507_4153	Cal7103DRAFT_00060540	164		TALAYLGDA	1	N	
			Cal7507_3430		227	ERLEFLGDA		1	Y	
					237	EQLEFVGDA		1	Y	
<i>Chroococcidiopsis</i> sp. PCC 6712	2	2,1	Chr6712_2695	Cal7507_1114	159		TALAYMGDA	1	N	
			Chr6712_3177		238	ERLEFLGDA		1	Y	
<i>Chroococcidiopsis thermalis</i> PCC 7203	2	2,1	Chro_4112	Chr6712_4648	244	EQLEFVGDA		1	Y	
			Chro_5889		152		VALAYIGDA	1	N	
					237	EQLEFVGDA		1	Y	
<i>Crocospaera watsonii</i> WH 8501	1	2,1	CWat_WH8501_draft2_00045690	Chro_4036	493	EWLALLGDT		2	N	Gun4-like
			CWat_WH8501_draft2_00017180		169		MALAYLGDA	1	N	
					238	QQLEFVGDS		1	Y	
<i>Cyanobacterium</i> PCC 7702	5	2,1	Ch17702DRAFT_0536	CWat_WH8501_draft2_00007740	328	QRLESLGEN		1	N	
			Ch17702DRAFT_2265		137		AFLAYIGDA	1	N	
					225	ERLEFLGDA		1	Y	
<i>Cyanobacterium</i> sp. UCYN-A	1	1,1	UCYN_03680	Ch17702DRAFT_2383	244	EHLEFVGDA		1	Y	
					160		TTLAYLGDA	1	N	
<i>Cyanothece</i> sp. BH68, ATCC 51142	1	1,1	cce_3135	UCYN_00750	243	QQLEFVGDS		1	Y	
					137		AFLAYIGDA	1	N	
<i>Cyanothece</i> sp. PCC 7424	1	2,1	PCC7424_3959	cce_1800	242	QQLEFVGDS		1	Y	
			PCC7424_2664		137		AFLAYIGDA	1	N	
<i>Cyanothece</i> sp. PCC 7425	1	1,1	Cyan7425_0389	PCC7424_3194	154		ASLAYIGDA	1	N	
					238	EQLEFVGDA		1	Y	
<i>Cyanothece</i> sp. PCC 7822	1	2,1	Cyan7822_0327	Cyan7425_1940	153		AALAYLGDA	1	N	
			Cyan7822_1001		228	ERLEFLGDA		1	Y	
<i>Cyanothece</i> sp. PCC 8801	1	3,1	PCC8801_3303	Cyan7822_0774	235	QQLEFVGDA		1	Y	
			PCC8801_2820		131		ASLAYIGDA	1	N	
			PCC8801_0645		225	ERLEFLGDA		1	Y	
<i>Dactylococcopsis salina</i> PCC 8305	1	2,1	Daesa_1083	PCC8801_2425	236	QQLEFLGDS		1	Y	
			Daesa_3634		323	ERLEFLGDA		1	N	
					158		AFLAYLGDA	1	N	
<i>Fischerella</i> sp. PCC 9339	5	2,1	PCC9339DRAFT_01064	Daesa_2494	237	ERLEFLGDA		1	Y	
			PCC9339DRAFT_03154		264	QQLEFVGDA		1	Y	
					150		AALAYIGDA	1	N	
<i>Fischerella</i> sp. PCC 9431	5	2,1	Fis9431DRAFT_3759	PCC9339DRAFT_04090	224	ERLEFLGDA		1	Y	
			Fis9431DRAFT_1534		239	EHLEFVGDA		1	Y	
					160		VALAYLGDA	1	N	
<i>Fischerella</i> sp. PCC 9605	5	2,1	FIS9605DRAFT_02642	Fis9431DRAFT_4808	160		VALAYLGDA	1	N	
			FIS9605DRAFT_04201		239	EHLEFVGDA		1	Y	
					232	ERLEFLGDA		1	Y	
<i>Geitlerinema</i> sp. PCC 7105	3	2,0	Gei7105DRAFT_1223	FIS9605DRAFT_03180	243	EHLEFVGDA		1	Y	
			Gei7105DRAFT_5151		159		TALAYLGDA	1	N	
<i>Geitlerinema</i> sp. PCC 7407	3	2,1	GEI7407_1007		247	EQLEFVGDA		1	Y	
			GEI7407_0526		366	ERLEFLGDS		1	Y	
					231	ERLEFLGDA		1	Y	
<i>Gloeobacter violaceus</i> PCC 7421	1	1,1	gvip371	GEI7407_3792	246	EQLEFMGDA		1	Y	
					167		VALAYLGDA	1	N	
<i>Leptolyngbya boryana</i> PCC 6306	3	1,1	LepboDRAFT_1185	glr4180	106	DRLEFLGDE		1	Y	
					242		VALAYLGDA	1	N	
<i>Leptolyngbya</i> sp. PCC 6406	3	3,1	LEP6406DRAFT_2207	LepboDRAFT_4252	238	EQLEFVGDA		1	Y	
			LEP6406DRAFT_1871		139		AAFAYLGDA	1	N	
			LEP6406DRAFT_1169		133	ERLEFVGDS		1	N	
<i>Leptolyngbya</i> sp. PCC 7375	3	3,1	Lepto7375DRAFT_7811	LEP6406DRAFT_3537	224	ERLEFLGDA		1	Y	
			Lepto7375DRAFT_5336		254	EQLEFLGDA		1	Y	
			Lepto7375DRAFT_3393		166		QALAYIGDA	1	N	
<i>Leptolyngbya</i> sp. PCC 7376	3	2,1	Lepto7376_1012	Lepto7375DRAFT_2718	154		IALAYIGDA	1	N	
			Lepto7376_0760		153	EIFRTLGDA		1	N	
					223	ERLEFLGDA		1	Y	
<i>Mastigocladopsis repens</i> PCC 10914	5	2,1	Mas10914DRAFT_2231		256	EVLEFLGDA		1	Y	
			Mas10914DRAFT_2728		241	EVLEFLGDA		1	Y	
					258	ERLEFLGDA		1	Y	
<i>Microcoleus vaginatus</i> FGP-2	3	3,1	MicvaDRAFT_0740	Lepto7376_3431	129		TALAYVGDA	1	N	
			MicvaDRAFT_1301		239	EHLEFVGDA		1	Y	
			MicvaDRAFT_0189		388	ERLEFLGDA		1	Y	
<i>Microcystis aeruginosa</i> NIES-843	1	2,1	MAE_31570	Mas10914DRAFT_3118	160		TALAYLGDA	1	N	
					235	EQLEFVGDA		1	Y	
					237	RLEFLGDA		1	Y	
					588	KRSLSLGGA		1	N	
					148		AAWAYLGDA	1	N	
					226	ERLEFLGDA		1	Y	

			<i>MAE_60800</i>			234	EQLEFVGDS		1	Y
				<i>MAE_28410</i>		138		ASLAYLGD	1	N
<i>Nostoc azollae</i> 0708	4	2,1	<i>Aazo_4580</i>			227	ERLEFLGD		1	Y
			<i>Aazo_3201</i>			237	EQLEFVGDS		1	Y
				<i>Aazo_2284</i>		159		SALAYLGD	1	N
<i>Nostoc punctiforme</i> PCC 73102	4	2,1	<i>Npun_R1331</i>			228	ERLEFLGD		1	Y
			<i>Npun_F1233</i>			239	EQLEFVGDS		1	Y
				<i>Npun_R1191</i>		159		TALAYLGD	1	N
<i>Nostoc sp.</i> PCC 7107	4	3,1	<i>Nos7107_4879</i>			227	ERLEFLGD		1	Y
			<i>Nos7107_2456</i>			234	EVLEFFGDS		1	Y
			<i>Nos7107_0948</i>			239	EQLEFVGDS		1	Y
				<i>Nos7107_3662</i>		160		AALAYIGD	1	N
<i>Nostoc sp.</i> PCC 7524	4	3,1	<i>Nos7524_1070</i>			228	ERLEFLGD		1	Y
			<i>Nos7524_4545</i>			234	EVLEFFGDS		1	Y
			<i>Nos7524_1546</i>			238	EQLEFVGDS		1	Y
				<i>Nos7524_3113</i>		159		AALAYVGD	1	N
<i>Oscillatoria nigro-viridis</i> PCC 7112	3	3,1	<i>Osc7112_2758</i>			230	ERLEFLGD		1	Y
			<i>Osc7112_3480</i>			234	EQLEFVGDS		1	Y
			<i>Osc7112_2759</i>			400	QLLEFLGD		1	Y
				<i>Osc7112_2944</i>		148		AAWAYLGD	1	N
<i>Pleurocapsa sp.</i> PCC 7319	2	3,1	<i>Pleur7313DRAFT_05633</i>			240	ERLEFLGD		1	Y
			<i>Pleur7313DRAFT_00733</i>			246	EQLEFVGDS		1	Y
			<i>Pleur7313DRAFT_05876</i>			319	QRLEFLGK		1	N
				<i>Pleur7313DRAFT_05600</i>		158		IALAYIGD	1	N
<i>Pleurocapsa sp.</i> PCC 7327	2	2,1	<i>Ple7327_4215</i>			228	ERLEFLGDS		1	Y
			<i>Ple7327_0296</i>			244	QQLEFVGDA		1	Y
				<i>Ple7327_2958</i>		152		IALAYIGD	1	N
<i>Prochlorococcus marinus</i> AS9601	1	1,1	<i>A9601_18131</i>			249	EKLEFFGDA		1	Y
				<i>A9601_09111</i>		132		IQLAWLGD	1	N
<i>Prochlorococcus marinus marinus</i> CCMP 1375	1	1,1	<i>Pro1762</i>			249	ERLEFQGD		1	Y
				<i>Pro0747</i>		137		LQLAWLGD	1	N
<i>Prochlorococcus marinus</i> MIT 9211	1	1,1	<i>P9211_17271</i>			249	ERLEFLGD		1	Y
				<i>P9211_06951</i>		144		LQLAWLGD	1	N
<i>Prochlorococcus marinus</i> MIT 9215	1	1,1	<i>P9215_18771</i>			249	EKLEFFGDA		1	Y
				<i>P9215_09411</i>		132		IQLAWLGD	1	N
<i>Prochlorococcus marinus</i> MIT 9301	1	1,1	<i>P9301_17961</i>			249	EKLEFFGDA		1	Y
				<i>P9301_09091</i>		132		IQLAWLGD	1	N
<i>Prochlorococcus marinus</i> MIT 9303	1	1,1	<i>P9303_26001</i>			262	ERLEFLGD		1	Y
				<i>P9303_15791</i>		135		LQLAWLGD	1	N
<i>Prochlorococcus marinus</i> MIT 9312	1	1,1	<i>PMT9312_1696</i>			249	EKLEFFGDA		1	Y
				<i>PMT9312_0850</i>		132		IQLAWLGD	1	N
<i>Prochlorococcus marinus</i> MIT 9313	1	1,1	<i>PMT1949</i>			281	ERLEFLGD		1	Y
				<i>PMT0651</i>		132		LQLAWLGD	1	N
<i>Prochlorococcus marinus</i> MIT 9515	1	1,1	<i>P9515_1791</i>			249	EKLEFFGDA		1	Y
				<i>P9515_10321</i>		130		IQLAWLGD	1	N
<i>Prochlorococcus marinus</i> NATL1A	1	1,1	<i>NATL1_20541</i>			247	ENLEFLGD		1	Y
				<i>NATL1_09301</i>		132		LQLAWLGD	1	N
<i>Prochlorococcus marinus</i> NATL2A	1	1,1	<i>PMN2A_1179</i>			247	ENLEFLGD		1	Y
				<i>PMN2A_0261</i>		132		LQLAWLGD	1	N
<i>Prochlorococcus marinus</i> pastoris CCMP 1986	1	1,1	<i>PMM1603</i>			249	EKLEFFGDA		1	Y
				<i>PMM0949</i>		130		IQLAWLGD	1	N
<i>Prochlorococcus sp.</i> CC931	1	1,1	<i>sync_0200</i>			254	EQLEFLGD		1	Y
				<i>sync_1634</i>		135		LQLAWLGD	1	N
<i>Prochlorococcus sp.</i> CC9605	1	1,1	<i>Syncc9605_0149</i>			241	ERLEFLGD		1	Y
				<i>Syncc9605_1154</i>		136		LQLAWIGD	1	N
<i>Prochlorococcus sp.</i> CC9902	1	1,1	<i>Syncc9902_0179</i>			249	ERLEFLGD		1	Y
				<i>Syncc9902_1305</i>		143		LQLAWIGD	1	N
<i>Prochlorococcus sp.</i> WH 7803	1	1,1	<i>SynWH7803_0204</i>			240	EQLEFLGD		1	Y
				<i>SynWH7803_1063</i>		136		LQLAWLGD	1	N
<i>Prochlorothrix hollandica</i> PCC 9006	3	2,1	<i>Pro9006DRAFT_0098</i>			242	EQLEFVGDA		1	Y
			<i>Pro9006DRAFT_0103</i>			256	ERLEFLGD		1	Y
				<i>Pro9006DRAFT_2926</i>		132		VLLAYVGD	1	N
<i>Pseudanabaena sp.</i> PCC 6802	3	2,1	<i>Pse6802_3090</i>			220	QRLEFLGDC		1	Y
			<i>Pse6802_2378</i>			245	DVLEFVGDS		1	Y
				<i>Pse6802_4789</i>		120		ASLAYLGD	1	N
<i>Pseudanabaena sp.</i> PCC 7367	3	2,1	<i>Pse7367_2482</i>			221	QRLEFLGD		1	Y
			<i>Pse7367_1487</i>			269	EKLEFFGDS		1	Y
				<i>Pse7367_2852</i>		132		AALAYIGD	1	N
<i>Spirulina major</i> PCC 6313	3	2,1	<i>Spi6313_3729</i>			235	ERLEFLGD		1	Y
			<i>Spi6313_2493</i>			239	EQLEFVGDA		1	Y
				<i>Spi6313_1300</i>		150		TALAYIGD	1	N
<i>Spirulina subsalsa</i> PCC 9445	3	4,1	<i>Spi9445_2508</i>			227	ERLEFLGD		1	Y
			<i>Spi9445_1629</i>			246	EQLEFVGDA		1	Y
			<i>Spi9445_4283</i>			311	ERLVVLGGM		1	N
			<i>Spi9445_1201</i>			377	ERLEFLGDT		1	Y
				<i>Spi9445_3328</i>		151		LALAYLGD	1	N
<i>Synechococcus elongatus</i> PCC 7942	1	1,1	<i>Syncc7942_1645</i>			247	DRLEFLGD		1	Y
				<i>Syncc7942_2120</i>		138		QALAYLGD	1	N
<i>Synechococcus sp.</i> PCC 7002	1	2,1	<i>SYNPCC7002_A0061</i>			239	EQLEFIGDA		1	Y
			<i>SYNPCC7002_A2542</i>			258	ERLEFLGD		1	Y
				<i>SYNPCC7002_A0384</i>		131		ASLAYVGD	1	N
<i>Synechocystis sp.</i> PCC 6803	1	2,1	<i>slr1646</i>			231	DRLEFLGD		1	Y
			<i>slr0346</i>			244	QQLEFVGDA		1	Y
				<i>slr0954</i>		143		VALAYLGD	1	N
<i>Thermosynechococcus elongatus</i> BP-1	1	0,1		<i>tlr0428</i>		151		AALAYFGD	1	N
<i>Trichodesmium erythraeum</i> IMS101	3	3,1	<i>Tery_2839</i>			209	QGLANLGYV		1	N
			<i>Tery_2144</i>			233	ESLEFIGDA		1	Y
			<i>Tery_1105</i>			250	EKLEFFGDA		1	Y
				<i>Tery_2312</i>		130		SAWAYLGD	1	N
<i>Arabidopsis thaliana</i>	N/A	N/A		<i>AT1G55140</i>		237		ASLAYIGDS	1	N
<i>Bacillus subtilis</i>	N/A	N/A		<i>gnt</i>		143		LALAYIGD	1	N
<i>Escherichia coli</i> MG1655	N/A	N/A	<i>rnc</i>			226	ERLEFLGDS		1	Y

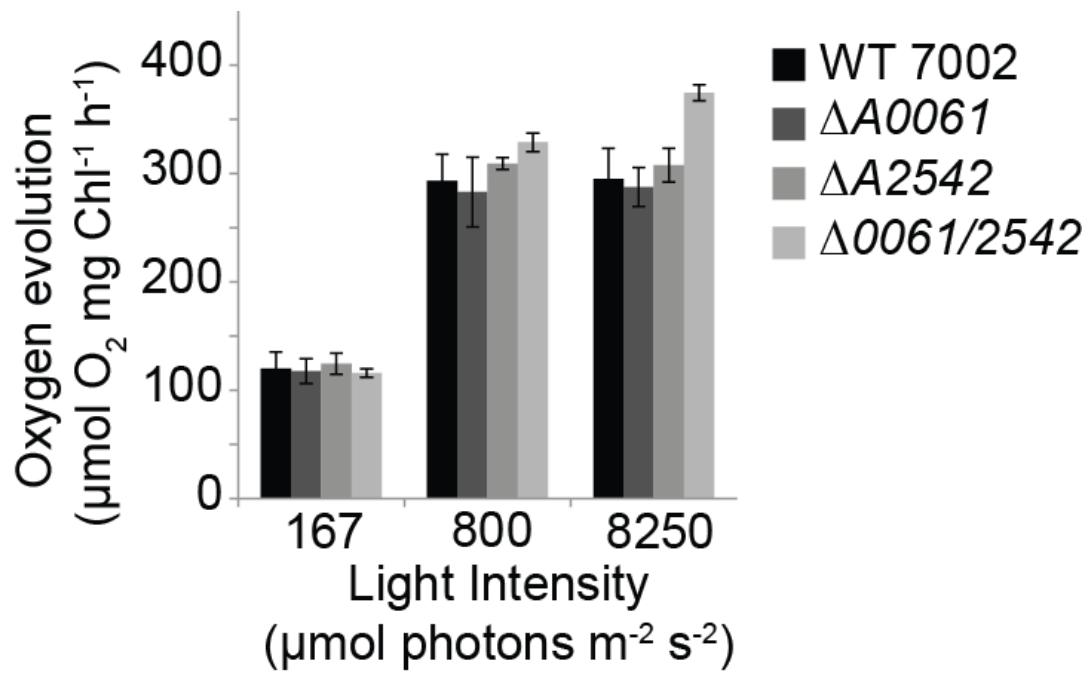
Model organisms used in Table I are highlighted in bold.

⁸Shih PM et al. (2013) Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. Proc Natl Acad Sci U S A. 110 (3): 1053-1058



Online Resource 5 Growth and physiology of $\Delta 1543$ strains

Growth of WT and $\Delta 1543$ strains in liquid medium (12-well tissue culture plates) with shaking (150 rpm) in the absence (a) and presence (b) of 10 mM NaHCO_3^- . Error bars are s.d. of three cultures. Cells were grown in standard conditions as described in “Materials and Methods”. (c) Absorption spectra of WT, $\Delta 1543$, and genetically complemented $\Delta 1543+$ strains following 24 h growth in liquid medium in culture tubes bubbled with air.



Online Resource 6 Light-dependent oxygen evolution activity of RNase III mutants

Oxygen evolution was measured as a function of light intensity on a Clark-type electrode in cell solutions suspended at a chlorophyll concentration of 5 $\mu\text{g/ml}$ in A+ media containing 10 mM NaHCO_3^- as described in “Materials and Methods”.