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Supplementary Information for

Comparative Genomics Reveals the Molecular Determinants of Rapid Growth of the Cyanobacterium *Synechococcus elongatus* UTEX 2973

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SI Methods

Construction of Plasmids. For all cyanobacterial strains (See SI Appendix, Table S1) and primers (See SI Appendix, Table S2), the following naming convention was used. Plasmids to target any given gene were constructed using primers that are named beginning with the M744_ locus tag. For example, primers used to make the editing vector to convert *Synechococcus* 2973 to the *Synechococcus* 7942 version of the M744_01335 gene were all named beginning with 1335. Below we refer to this number as XXXX when referring to primers. To construct an editing vector, pSL2680 was digested with AarI restriction enzyme. XXXXgRNAL and XXXXgRNAR oligos were annealed and phosphorylated such that 5' overhangs were produced to be compatible with the digested pSL2680 vector. Annealed oligos were ligated into pSL2680 replacing lacZ, which was removed by AarI digestion. Blue/white screening was used to identify those colonies with an insert after transformation. Correct colonies were also verified by Sanger sequencing of the plasmid using the repairseqR primer. Repair templates were synthesized as right and left halves via PCR with Phusion high fidelity polymerase (Thermo) using the XXXXL/XXXXMR and XXXXML/XXXXR primer pairs. To generate a repair template to mutate *Synechococcus* 2973 to the *Synechococcus* 7942 allele of a gene, we used *Synechococcus* 7942 chromosomal DNA as template because it already contained the desired change. The two PCR fragments overlapped by 40 nucleotides, centered at the PAM site as well as 30 nucleotides with the vector. An additional mutation was introduced via the primers during PCR amplification to create a silent mutation, removing the PAM on the repair template. Next, we digested the plasmid containing the annealed and ligated oligos with KpnI while dephosphorylating it with FastAP (Thermo). The two PCR fragments that comprise the repair template were then assembled into the vector using Gibson assembly. Finally, the repair template was verified to be free of unintended mutations using Sanger sequencing.

For overexpression of *ppnK* from either strain, we amplified *Synechococcus* 2973 or *Synechococcus* 7942 chromosomal DNA with the 4780hisL/4780Re primer pair. The resulting PCR product was then digested with NdeI and KpnI and ligated into pET44b (29) digested with the same enzymes, resulting in N-terminal His-tagged PpnK. The overexpression strain with the *Synechococcus* 2973 allele was named pSL3052 while the overexpression strain with the *Synechococcus* 7942 allele was named pSL3053.

Overexpression and purification of PpnK. For overexpression of the two variants of PpnK, overnight cultures of either pSL3052 or pSL3053 were diluted 1:100 into 200 mL LB supplemented with 100 μg/mL ampicillin and grown to an OD600 of 0.5-0.7 at which time protein expression was induced by the addition of 1 mM IPTG. The cultures were then grown overnight at room temperature to allow for low level protein expression. Cells were then harvested and resuspended in 20 mL lysis buffer (20 mM sodium phosphate buffer, pH 8, 100 mM NaCl, 10% glycerol, 1 mM b-mercaptoethanol and protease inhibitor cocktail). Cells were chilled on ice and disrupted with five 30s bursts of sonication with a probe sonicator. The lysate was then clarified by centrifugation for 30 min at 45,000 g at 4^{o}C. The lysate was transferred to a new tube, 4 mL 50% Ni-NTA resin slurry was added to the lysate and placed on a rocker at 4^{o}C for 2 hours. The resin was then loaded on a 5 mL column (Pierce) and the supernatant was removed via centrifugation at 500g for 1 minute at 4^{o}C. The column was then rinsed with 3 mL lysis buffer 5 times followed by 3 mL wash buffer (20 mM sodium phosphate buffer, pH 8, 100 mM NaCl, 10%

glycerol, 1mM b-mercaptoethanol, 10 mM imidazole) 5 times. Finally, the protein was eluted with 2 elutions of 3 mL elution buffer (20 mM sodium phosphate buffer, pH 8, 100 mM NaCl, 10% glycerol, 1 mM b-mercaptoethanol, 150 mM imidazole) and the two fractions were pooled. The protein was then concentrated and buffer exchanged with storage buffer (20 mM sodium phosphate buffer, pH 8, 100 mM NaCl, 10% glycerol, 1 mM b-mercaptoethanol) using 10K MWCO concentrator columns (Thermo). Protein concentration was adjusted to 1mg/mL for each sample using a Bradford assay. Purity of the sample was verified via SDS-PAGE. Aliquots of 25 μL were snap frozen and stored at -80^{o}C.

77K Fluorescence. Cultures were grown in MC-1000 multicultivators at 38^{o}C, 5% CO_2 and either 900 or 400 μmol m^{-2} s^{-1} light for *Synechococcus* 2973 and *Synechococcus* 7942 respectively. The fluorescence emission spectra of phycobilisomes from whole cells of each strain were measured at 77K with samples adjusted to equal cell number ($2x10^8$ cells per ml). Excitation occurred at 590 nm and fluorescence was recorded from 600 nm to 750 nm and normalized at 750 nm. The measurements were made on a SPEX fluoromax 2 spectrofluorometer and analyzed with Data Max for Windows.

Absorption Spectra. Cultures were grown in MC-1000 multicultivators at 38^{o}C, 5% CO_2 and either 900 or 400 μmol m^{-2} s^{-1} light for *Synechococcus* 2973 and *Synechococcus* 7942, respectively. Absorption spectra of *Synechococcus* 2973 and *Synechococcus* 7942 that were harvested during log phase were determined on an Olis DW-2000 spectrophotometer and data were analyzed with Olis Globalworks software. Spectra were normalized at 750 nm to correct for differences in light scattering.

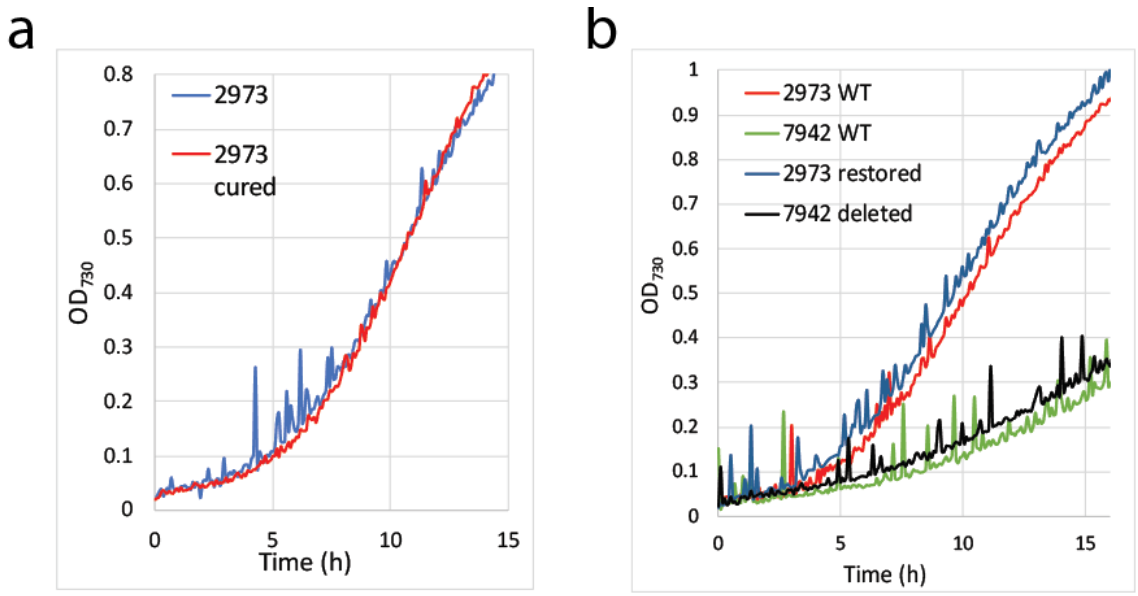


Fig. S1. Comparison of growth between *Synechococcus* 2973 and *Synechococcus* 7942 strains. (a) Wild type *Synechococcus* 2973 compared to the same strain after its small plasmid has been cured. (b) Growth of wild type *Synechococcus* 2973 compared to the same strain with the 7-kb deletion restored and growth of wild type *Synechococcus* 7942 compared to the same strain with the 7-kb deletion incorporated**.**

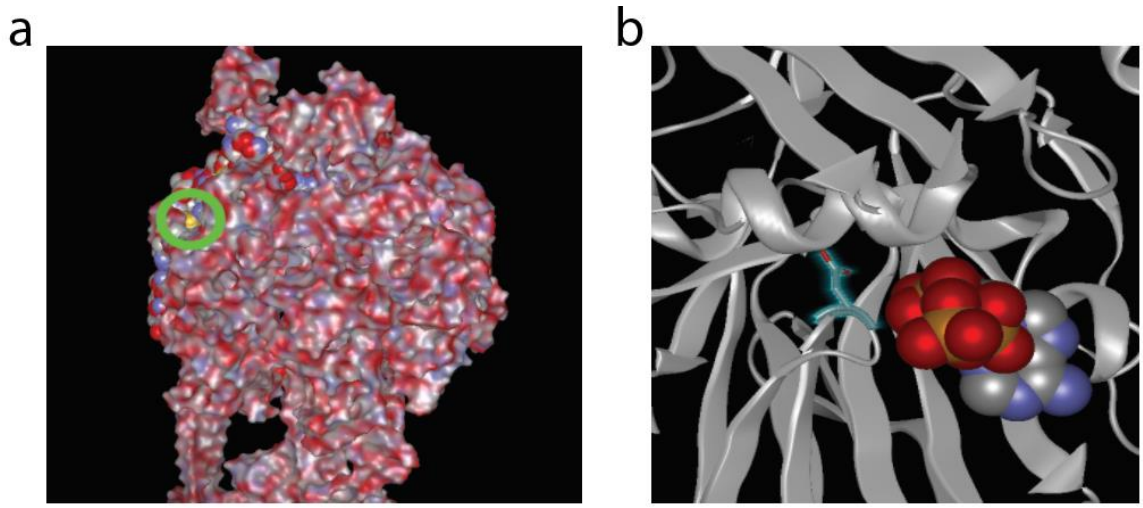


Fig. S2. Predicted structures of ATP synthase and NAD^+ kinase from *Synechococcus* strains. (a) Surface map of ATP synthase from *Synechococcus* 7942. Red: negative charge, blue: positive charge. Yellow:C252 on the surface circled in green. (b) Local ribbon diagram of NAD^+ kinase active site from *Synechococcus* 2973 associated with ATP. E260, the amino acid changed by the SNP, is the only amino acid whose side chain is depicted in detail. Additionally, this amino acid is highlighted in blue. ATP is shown as space filling. Red: oxygen, yellow: phosphorus, blue: nitrogen, grey: carbon.

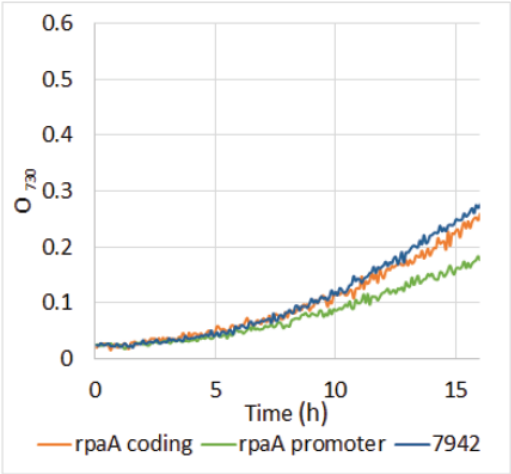
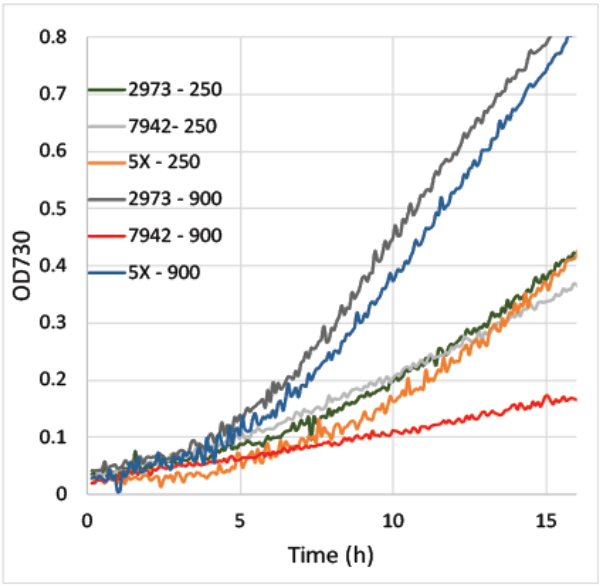


Fig. S3. Growth of *Synechococcus* 7942 and *Synechococcus* 7942 with the pair of coding SNPs or the promoter SNP from *Synechococcus* 2973.

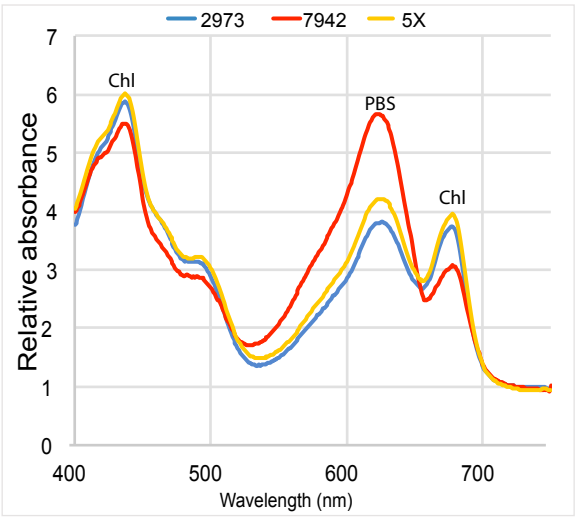
a



b

	Doubling time (h)
2973 -250	4.22
7942 -250	4.38
5X - 250	3.77
2973- 900	2.26
7942 -900	6.93
5X -900	2.30

c



d

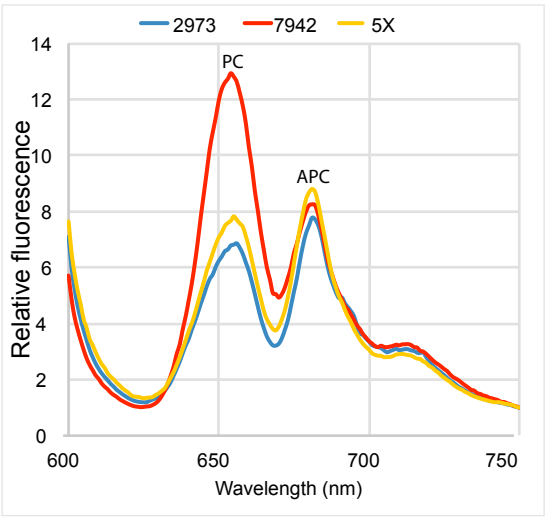


Fig. S4. Physiological Comparison of *Synechococcus* 7942 and *Synechococcus* 2973. (a) Representative growth of wild type *Synechococcus* strains and *Synechococcus* 7942 with 3 alleles converted to the *Synechococcus* 2973 version (5X). (b) Doubling times calculated from (a). Numbers indicate light intensity in µmol m^{-2} s^{-1}. (c) Absorbance scan of either wild type or the 5X mutant normalized at 750nm. (d) 77K fluorescence emission spectra of either wild type or the 5X mutant normalized at 750nm. Chl, chlorophyll; PBS, phycobilisomes; PC, phycocyanin; APC, allophycocyanin.

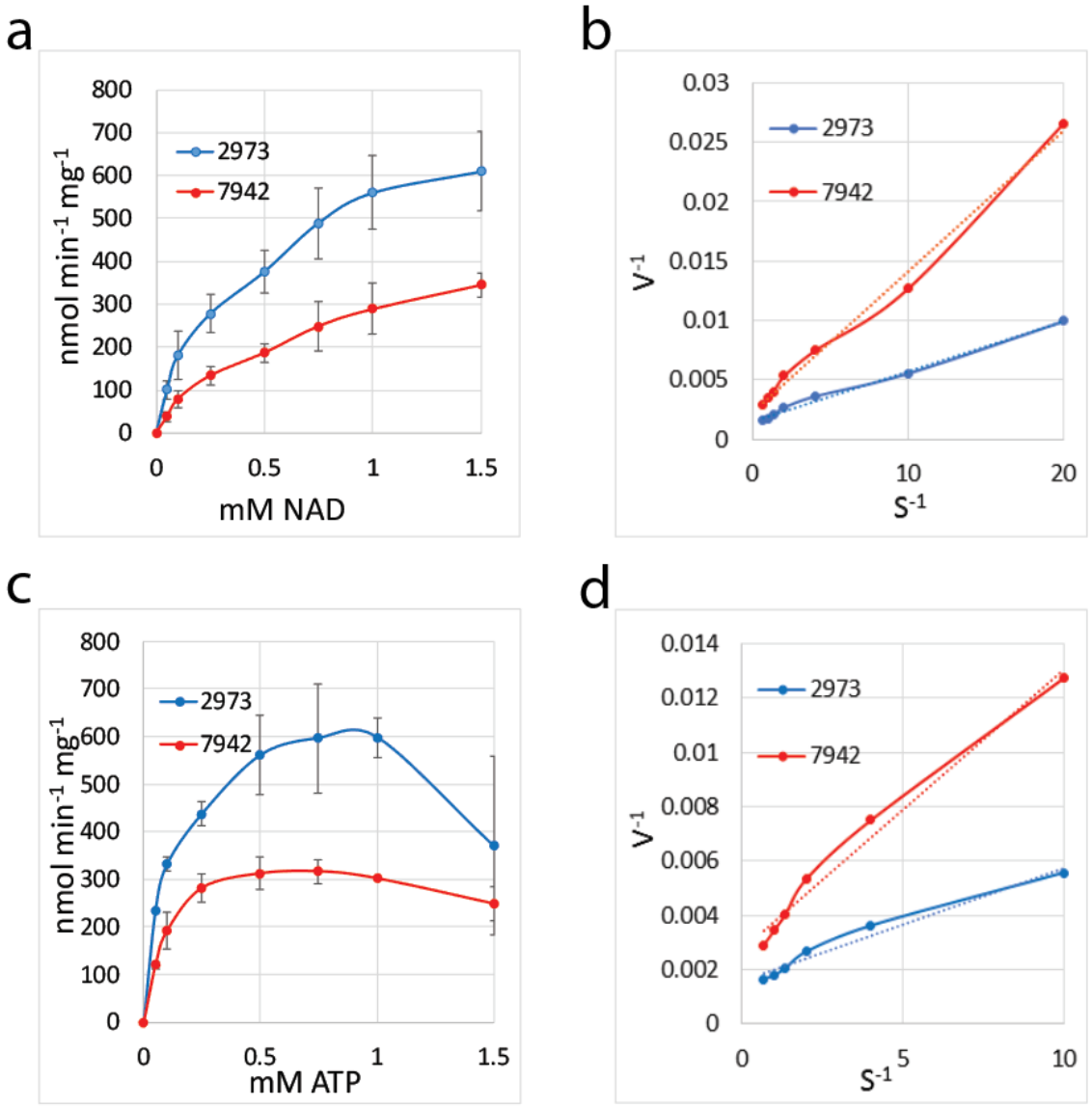
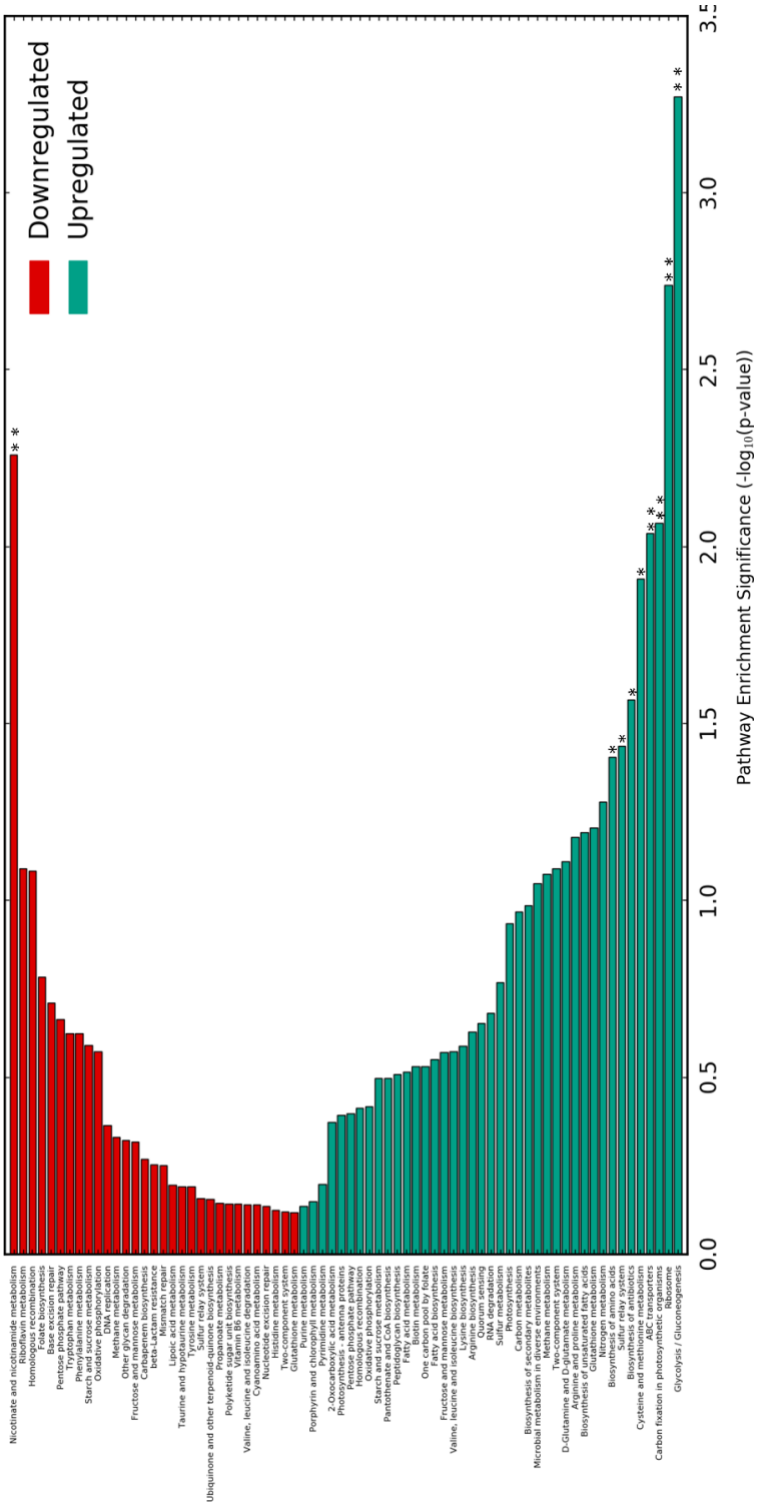


Fig. S5. Enzyme kinetics of the *Synechococcus* 7942 and *Synechococcus* 2973 alleles of NAD^+ kinase. (a) Michaelis-Menten plot of NAD^+ kinase activity at various NAD concentrations. (b) Lineweaver-Burke plot of NAD^+ kinase activity at various NAD concentrations. (c) Michaelis-Menten plot of NAD^+ kinase activity at various ATP concentrations. (d) Lineweaver-Burke plot of NAD^+ kinase activity at various ATP concentrations (n=3).

Fig.



S6. Bar graph comparing the -log10(p-value) of individual pathways. The data from the analysis of the upregulated and down regulated genes are combined in one plot. The green bars correspond to the pathways represented among the upregulated genes and the red bars correspond to pathways overrepresented among the down regulated genes. *indicates p-value <0.05 and ** indicates p-value <0.01. Upregulated and downregulated refer to those in *Synechococcus* 2973 when compared against *Synechococcus* 7942.

Table S1: Cyanobacterial Strains

Reversions of *Synechococcus* 2973 coding SNPs to *Synechococcus* 7942 alleles

Locus Tag	Annotation	Strain Name
M744_705	Hypothetical protein	SL2820
M744_1335	ATP synthase F0F1 subunit alpha	SL2770
M744_2605-1	RpaA, Circadian Regulator	SL2790
M744_2605-2	RpaA, Circadian Regulator	SL2790
M744_3335	Manganese ABC transporter ATP-binding protein	SL2849
M744_3855	Guanylate cyclase	SL2821
M744_4780	PpnK	SL2848
M744_5865	Hypothetical protein	SL2822
M744_6025	Molecular chaperone DnaK	SL2791
M744_6570-1	Hydrolase	SL2792
M744_6570-2	Hydrolase	SL2792
M744_6650-1	CTP synthetase	SL2850
M744_6650-2	CTP synthetase	SL2856
M744_6850	Chorismate mutase	SL2811
M744_8615	DNA-directed RNA polymerase ß subunit	SL2768
M744_11685	Anthranilate synthase	SL2803
M744_12130-1	Long-chain-fatty-acid CoA ligase	SL2916
M744_12130-2	Long-chain-fatty-acid CoA ligase	SL2815
M744_12285	Glutamate synthase	SL2771
M744_13540	Photosystem I assembly protein Ycf4	SL2755

Reversions of *Synechococcus* 2973 noncoding SNPs to *Synechococcus* 7942 alleles

Nucleotide Position		
474883	Missing: ACTATCAT	SL2942
1042862	T:C	SL2943
1533430	G:A	SL2965
1741647	C:A	SL2966
2139224	Missing: T	SL2944
2347222	A:G	SL2939
2348957	G:T	SL2941
2347664-2347720	Insertion 56 nucleotides	SL2940
474883 with 2605 1&2	Combined SL2942 and SL2790 in one strain	SL2942-2

Introduction of *Synechococcus* 2973 SNPs into *Synechococcus* 7942

M744_1335	ATP synthase F0F1 subunit alpha	SL2851
M744_2605-1	Chemotaxis protein CheY	SL2982
M744_2605-2	Chemotaxis protein CheY	SL2982
M744_4780	PpnK	SL2860
NC-474883	RpaA - two component transcriptional regulator, winged helix family	SL2993
2X	SL2851 and SL2860 combined in one strain	SL2860-2
2X	SL2993 and SL2982 combined in one strain	SL2993-2
5X	SL2851, SL2860, SL2982, SL2993 combined in one strain	SL2993-3
474883 with 2605 1&2	SL2982 and SL2993 combined in same strain	SL2993-4
	cure 2973 of plasmid	SL2812
	Reintroduction of genes in deletion into *Synechococcus* 2973	SL2923
	Creation of 7 kb deletion in *Synechococcus* 7942	SL3001

Table S2: Primers used in this study

Primer name	**Sequence** (5' to 3')
repair seqR	cgctgcccggattacagatc
8615gRNAL	agat CTCGCAACTCTCGCAGTTCA
8615gRNAR	agac TGAACTGCGAGAGTTGCGAG
8615L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GCCCCTGATGACTGATCGCG
8615ML	CAAAGAATTCTTTGGaTCCTCGCAACTCTCGC
8615MR	GCGAGAGTTGCGAGGAtCCAAAGAATTCTTTG
8615R	cgctgcccggattacagatcctctagagtcgacGGTACC GTGGCAGAACCGTCAGCAATC
8615seqM	GCTGGAATCGCGGTTCTTCG
13540gRNAL	agat ACAGCAGCCAATCTTGGGAT
13540gRNAR	agac ATCCCAAGATTGGCTGCTGT
13540L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GTACGGACGGACGCCCACC
13540ML	GTTGAGACGGATTCaTTACAGCAGCCAATCTTGGGAT
13540MR	ATCCCAAGATTGGCTGCTGTAAtGAATCCGTCTCAAC
13540R	cgctgcccggattacagatcctctagagtcgacGGTACC GCTGGTGCCGTCGTAAACTC
13540seqM	CACGACCTAAGATCGAGACCG
1335gRNAL	agatCCGCCAGATGTCGCTGCTGC
1335gRNAR	agacGCAGCAGCGACATCTGGCGG
1335L	ggtcatttttttgtctagctttaatgcggtagttGGTACC CATTCGTCAGCAGATTGAGCAG
1335ML	CCAAGCAAGCGCAGGCgTACCGCCAGATGTCGC
1335MR	GCGACATCTGGCGGTAcGCCTGCGCTTGCTTGG
1335R	cgctgcccggattacagatcctctagagtcgacGGTACC CGACTAGACAGCAGCCAAGATG
1335seqM	GCCTCTTCGGTTGCCAAC
12285gRNAL	agatCACCACCGCCTCACCACGAC
12285gRNAR	agacGTCGTGGTGAGGCGGTGGTG
12285L	catttttttgtctagctttaatgcggtagttGGTACCGTCGAC GCTACCCAGCTATCACGGTG
12285ML	GGTGTCTCGCTAATaTCACCACCGCCTCACCACGAC
12285MR	GTCGTGGTGAGGCGGTGGTGAtATTAGCGAGACACC
12285R	ccgttgcgctgcccggattacagatcctctagaGTCGAC GTTGACGATCGCTTGCTCG
12285seqM	CTCGCGATCGCCATGAACC
2885gRNAL	agatTCATTATCGTTGGCATCTTG
2885gRNAR	agacCAAGATGCCAACGATAATGA
2885L	ggtcatttttttgtctagctttaatgcggtagttGGTACC CCGTCGAAGCATCATCAACAATG
2885ML	CGAGTTGCTGGTCGTaATCATTATCGTTGGCATC
2885MR	GATGCCAACGATAATGATtACGACCAGCAACTCG
2885R	cgctgcccggattacagatcctctagagtcgacGGTACC GCAGATAACGTAGACGGAGCG
2885seqM	CCAATGCGGACGGAGCCAAAG
3975gRNAL	agat AGGTCTTCCCGCAGGTCAAA
3975gRNAR	agac TTTGACCTGCGGGAAGACCT
3975L	ggtcatttttttgtctagctttaatgcggtagttGGTACC CCAGTACGGATTGCAAGAAGC
3975ML	CACGTCCTTAATaCAGGTCTTCCCGCAGGTCAAATC
3975MR	GATTTGACCTGCGGGAAGACCTGtATTAAGGACGTG
3975R	cgctgcccggattacagatcctctagagtcgacGGTACC GGACTGCAGCTGCAAGCCAG
3975seqM	CTGATGTGGGCGTGGTGTTGG
2605gRNAL	agat CCCATGCAGCCCGCCATAGC
2605gRNAR	agac GCTATGGCGGGCTGCATGGG

2605L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GCCCTGCGTCTCAATTAATCTCC
2605ML	GCTGCAGCGCACCGATCGCATaCCCCATGCAGCCCGCCATAGC
2605MR	GCTATGGCGGGCTGCATGGGGtATGCGATCGGTGCGCTGCAGC
2605R	cgctgcccggattacagatcctctagagtcgacGGTACC GAGTCCTGAGCTGCTACTGCC
2605seqM	GTTTGTCCTCCCGGAATGTTACC
6025gRNAL	agat CGATTTTGCAGCTCGGCGAT
6025gRNAR	agac ATCGCCGAGCTGCAAAATCG
6025L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GTTTGAAGCGTATCGGCGTC
6025ML	GGGCAGCACCCTCGATGTaTCGATTTTGCAGCTCGGCGATA
6025MR	TATCGCCGAGCTGCAAAATCGAtACATCGAGGGTGCTGCCC
6025R	cgctgcccggattacagatcctctagagtcgacGGTACC GAATGCCGTCAGCATCAATATCG
6025seqM	CCGCGATCGCGTCTGTACCC
6570gRNAL	agat GGATTGCGGCCCAGATCATC
6570gRNAR	agac GATGATCTGGGCCGCAATCC
6570L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GTAACTGGGTCGCCGTCATGG
6570ML	CACGAGGCGAAGGACGCGATaTGGATTGCGGCCCAGATCATC
6570MR	GATGATCTGGGCCGCAATCCAtATCGCGTCCTTCGCCTCGTG
6570R	cgctgcccggattacagatcctctagagtcgacGGTACC CTTATACCAAGCAGCTGAACCTGC
6570seqM	CCTAGAAGGGGTGGATGCAATC
6650gRNAL	agat ACTGGGCACGTAATGTGGCA
6650gRNAR	agac TGCCACATTACGTGCCCAGT
6650L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GGTGACTATAACGGTGGCACG
6650ML	CTAGGGATGCAAGCAGCGGTGATaGACTGGGCACGTAATGTGGCAG
6650MR	CTGCCACATTACGTGCCCAGTCtATCACCGCTGCTTGCATCCCTAG
6650R	cgctgcccggattacagatcctctagagtcgacGGTACC CGCTTTAATCCTCGTAGGTCGCC
6650seqM	GGTCGATGCGGAAGATCTCG
12130gRNAL	agat GATCTATACCTCGGGCACCA
12130gRNAR	agac TGGTGCCCGAGGTATAGATC
12130gRNAL2	agatCTCACGGCAACCTGCTGCAC
12130gRNAR2	agacGTGCAGCAGGTTGCCGTGAG
121230L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GAGTGACTGGAACCGCCCTC
12130MR	TCGATCGCTTAGCCACaTTGATCTATACCTCGGGCACCAC
12130ML	GTGGTGCCCGAGGTATAGATCAAtGTGGCTAAGCGATCGA
12130R	cgctgcccggattacagatcctctagagtcgacGGTACC CGGTCTTGTCCCACCAACATG
12130seqM	CCACCGAAGGGCGTGATGC
11685gRNAL	agat CAACGGATCTGCTGGCTGAT
11685gRNAR	agac ATCAGCCAGCAGATCCGTTG
11685L	ggtcatttttttgtctagctttaatgcggtagttGGTACC GGTTGCGATCCACTCTGGGTG
11685ML	GCACCAGAAGATGCCGCCCTaGCAACGGATCTGCTGGCTGATCC
11685MR	GGATCAGCCAGCAGATCCGTTGCtAGGGCGGCATCTTCTGGTGC
11685R	cgctgcccggattacagatcctctagagtcgacGGTACC GCGATCGCAGAGCAATTCATTCC
11685seqM	CGACTGGCAGTTGATCGGCTC
6850gRNAL	agat AAGTGGCGATCGTGGGCAAGT
6850gRNAR	agac ACTTGCCCACGATCGCCACTT
6850L	ggtcatttttttgtctagctttaatgcggtagttGGTACC CAAGTCGCGGGGCTATTCGG
6850ML	CATCGCTAGAACTCAGGTTAcGaTCcGAcCAtGCAATGACTTAGACCTG
6850MR	CAGGTCTAAGTCATTGCaTGgTCgGAtCgTAACCTGAGTTCTAGCGATG

6850R	cgctgcccggattacagatcctctagagtcgacGGTACC CGGAAAGACGGGTGCAGATC
6650gRNAL2	agat TCGAGCGTCCTGATCATCCC
6650gRNAR2	agac GGGATGATCAGGACGCTCGA
6650L2	ggtcatttttttgtctagctttaatgcggtagttGGTACC CAAGTCGCGGGGCTATTCGG
6650ML2	CAGCCACTTGGTGGAGATaGTCGAGCGTCCTGATCATCCC
6650MR2	GGGATGATCAGGACGCTCGACtATCTCCACCAAGTGGCTG
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6850seqM	GTGCAGCAAATGTATGTGGAGG
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4780L	ggtcatttttttgtctagctttaatgcggtagttGGTACC CAGCAAACCGAGAGCCTGC
4780ML	GCCGGTTGCTATGTaTGGCCAGAAGAGCGGGTGTTAATC
4780MR	GATTAACACCCGCTCTTCTGGCCAtACATAGCAACCGGC
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3335gRNA-R	agac CAAGCGCGGATcCCCCTGAG
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3335-MR	GACCAAGCGCGGATGCCCCTGAGCtATCACACGCTGATTCAGCAG
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131199MR	caatcaaggtgatgatgaagatggtgtctgtgatacctacactctcatctctatgcagttttcaagg
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13203gRNAR	agac cacgcggtccaggttgcttg
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13203R	cgctgcccggattacagatcctctagagtcgacGGTACC ccagctgactatctcaatgatgctg
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13206ML	ggctcactctccctctcttaaggataacttattcgccccgg
13206R	cgctgcccggattacagatcctctagagtcgacGGTACC gtggcacagtcgatccaacag
13206seqM	ggctaacaggctagcagcgg
13206chrR	gcccagactcagacaacaacc
13204gRNAL	agat gggctgcctaatgtcacgtc
13204gRNAR	agac gacgtgacattaggcagccc
13204L	ggtcatttttttgtctagctttaatgcggtagttGGTACC gttcggtgtttggctagtgctg
13204MR	TCACGCTGCCGTTATTCCTTATTCTTCTTCGAAGTTCGCAAC
13204ML	GTTGCGAACTTCGAAGAAGAAT AAGGAATAACGGCAGCGTGA
13204R	cgctgcccggattacagatcctctagagtcgacGGTACC tggaactccagtcaacttctcactg
13204seqM	ctagacgattacccggtcgatc
13204chrR	cacaaggcggaggtagcttc
131058gRNAL	agat atccgctgtaagccgctgcc
131058gRNAR	agac ggcagcggcttacagcggat
131058L	ggtcatttttttgtctagctttaatgcggtagttGGTACC gttgtcagcaaggattccgagg
131058MR	cagcggatctatttatgttttcttaaaaATGATAGTggctctgttctggtgag
131058ML	ctcaccagaacagagccACTATCATttttaagaaaacataaatagatccgctg
131058R	cgctgcccggattacagatcctctagagtcgacGGTACC gcgcagatgacggatgtgg
131058seqM	ctaaaaccgcaaagctcggac
131058chrR	gttttgatgtagcgcgggtgc
131660gRNAL	agat atcttctttggctctgtcgc
131660grRNAR	agac gcgacagagccaaagaagat
131660L	ggtcatttttttgtctagctttaatgcggtagttGGTACC gagccgcttcatgtgcctg
131660MR	caaagaagatataccttctcagttgggtaggagcagtgtttg
131660ML	caaacactgctcctacccaactgagaaggtatatcttctttg
131660R	cgctgcccggattacagatcctctagagtcgacGGTACC catgagcacgcagggttcg
131660seqM	ccagacccagaaagctcctg
131660chrR	gggatggctaataaattgcggaag
132145gRNAL	agat ggtcgccagagatattaaga
132145gRNAR	agac tcttaatatctctggcgacc
132145L	ggtcatttttttgtctagctttaatgcggtagttGGTACC ggttcagtcgattagatcaactaatgcc
132145MR	cttcttaatatctctggcgactatataaagacagcctgatattttcc
132145ML	ggaaaatatcaggctgtctttatatagtcgccagagatattaagaag
132145R	cgctgcccggattacagatcctctagagtcgacGGTACC ctgcaacgaaggtgcggcg
132145seqM	ggtcacctaagaacgaactgacc
132145chrR	caaagctggcggcaagcg

132343gRNAL	agat aatccttttagtgtcaaat
132343gRNAR	agac atttgacactaaaaaggatt
132343L	ggtcatttttttgtctagctttaatgcggtagttGGTACC gtaagcgcccatcaactgcc
132343R	cgctgcccggattacagatcctctagagtcgacGGTACC ctaaatgaagggcgatcgcacc
132343MR	gagaaaatttttacactaaaaaggatttcagaatatgaggattc
132343ML	gaatcctcatattctgaaatccttttagtgtaaaattttctc
132343seqM	cgttttagctctgggcgaactttaac
132343chrR	ggaggaaaagagcaccaccaac
132721gRNAL	agat ccctgtttagatagagggct
132721gRNAR	agac agccctctatctaaacaggg
132721L	ggtcatttttttgtctagctttaatgcggtagttGGTACC ccgagtagcaaaacttcgcgg
132721MR	ggacacctaagaatagggcagcgatagccctctatctaaacagggttaagcctgttcgg
132721ML	ccgaacaggcttaaccctgtttagatagagggctatcgctgccctattcttaggtgtcc
132721R	cgctgcccggattacagatcctctagagtcgacGGTACC ccagaccttgataggcgatcg
132721seqM	cgcttgggagctgaaactcg
132721chrR	ctgcttgggcatcggcgatc
4780ML-2973	GCCGGTTGCTATGTaTGGCCAGAAGAcCGGGTGTTAATC
4780MR2-2973	GATTAACACCCGgTCTTCTGGCCAtACATAGCAACCGGC
2605-2973ML	ccttgctgcggcgcaccgatcgcataccccatgcagcccgccatagc
2605-2973MR	gctatggcgggctgcatggggtatgcgatcggtgcgccgcagcaagg
131058-2973ML	ctcaccagaacagagccttttaagaaaacataaatagatccgctg
131058-2973MR	cagcggatctatttatgttttcttaaaaggctctgttctggtgag
2605chrL	CTTGAGCTTTGCCGATGAAG
2605chrR	CGGTCAAGCCACGATTATCC
6025chrL	GGCTCTACAAGCAACTGGTCG
6025chrR	CAATTTCATCGCTGGTTAAGCCG
6570chrL	GCTCACGGCGGTGTTGTGG
6570chrR	GCGTTCAAAGCAACGGTAATCTC
6650chrL	GGCTCGATCTACCAGGCGG
6650chrR	CAATAATTGCCTCCGTCAGCAGC
8615chrL	GGCGAAATCAAGGAGCAGG
8615chrR	CATGTAGGCGACGAGGATG
1297chrL	CTCTGGGCGAATCCGAGTGG
1297chrR	CCGGCGCTACACGAGAATC
12130chrL	GAGGACGCTGGCTGAGGAG
12130chrR	CTGGCCCAGAGTGCGATCG
11685chrL	GGTGAGCACCTGGGTCGC
11685chrR	CGTCCCGAAGTAAGCGATCG
6850chrL	CCTTCAAGGTGATCAAGTCTGGC
6850chrR	GTACATCGGGTCTGCGCCTG
4780chrL	GGCGATGGTCGATGTGGCTC
4780chrR	CGATTTGACGACCATTACGAGC
0705chrL	CGGATACAGCACTGTCACGAAC
0705chrR	GATCTTCTAAGGACTCCTCAGCC
3335chrL	GCGGCTCTGACGATCCTG
3335chrR	GGCAAATCCCAGAAGAAGCTGAG
3855chrL	CGACGATTGGCAAACTCGC

3855chrR	CGTATTCCGAGTAAAACAGCGTGG
5865chrL	CTGCTCTGGGCTTAGATCTGG
5865chrR	GATCGCTGGGGACTCTCGC
4780 hisL	TATACATATGGGCAGCAGCCATCACCACCATCACCATTCTAGTGTGCCAGATGTTGGCATTATCTAC
4780Re	aataGGTACCGCAGTCTCAGTAAATGAGTGTGC

Datasets

Dataset S1: Loci identified to have >1.5-fold bidirectional change with allele substitutions via RNA-Sequencing

Dataset S2: Enrichment Analysis Output

Dataset S3: Comparison of transcriptomes of wild type *Synechococcus* 2973 to wild type *Synechococcus* 7942

Dataset S4: Genes identified to show significant changes in expression in a bidirectional comparison between *Synechococcus* 2973 and *Synechococcus* 2973 with the *Synechococcus* 7942 allele of ATP Synthase AND *Synechococcus* 7942 and *Synechococcus* 7942 with the *Synechococcus* 2973 allele of ATP Synthase

Dataset S5: Genes identified to show significant changes in expression in a bidirectional comparison between *Synechococcus* 2973 and *Synechococcus* 2973 with the *Synechococcus* 7942 allele of NAD+ Kinase AND *Synechococcus* 7942 and *Synechococcus* 7942 with the *Synechococcus* 2973 allele of NAD+ Kinase

Dataset S6: Genes identified to show significant changes in expression in a bidirectional comparison between *Synechococcus* 2973 and *Synechococcus* 2973 with the *Synechococcus* 7942 allele of *rpaA* AND *Synechococcus* 7942 and *Synechococcus* 7942 with the *Synechococcus* 2973 allele of *rpaA*

Dataset S7: A comparison of the alterations in gene expression that occurred as multiple alleles from *Synechococcus* 2973 were incorporated into *Synechococcus* 7942. In a separate tab, A comparison of the alterations in gene expression that occurred as multiple alleles from *Synechococcus* 7942 were incorporated into *Synechococcus* 2973