```
                    ┌─────────────────────┐
                    │  Sample Collection  │
                    └─────────────────────┘
                       │                │
                       ▼                ▼
        ┌──────────────────┐   ┌──────────────────────┐
        │ Culture Samples  │   │ Environmental Samples │
        │ Synechococcus    │   │ 1L seawater at BATS   │
        │ WH7803           │   └──────────────────────┘
        └──────────────────┘              │
                 │                         ▼
        ┌──────────────────┐   ┌──────────────────────┐
        │ Cell dilutions   │   │ Cell concentrations  │
        │ (10⁷ to 10³ cells)│  │ (From 1L to 5ml)     │
        └──────────────────┘   └──────────────────────┘
                 │                         │
                 │              ┌──────────────────────┐
                 │              │ Cell sorting         │
                 │              │ (~10⁶ cells)         │
                 │              └──────────────────────┘
                 ▼                         ▼
              ┌──────────────────────────────┐
              │          Cell lysis          │
              └──────────────────────────────┘
                 │              ╲           │
                 ▼               ╲          ▼
        ┌──────────────────┐   ┌──────────────────────┐
        │ MDA+ Illumina    │   │ Transposon-based     │
        │ library          │   │ library              │
        └──────────────────┘   └──────────────────────┘
                 │                         │
                 ▼                         ▼
              ┌──────────────────────────────┐
              │      Illumina Sequencing     │
              └──────────────────────────────┘
                 │                         │
                 ▼                         ▼
              ┌──────────────────────────────┐
              │         Data Analysis        │
              └──────────────────────────────┘
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Sample Collection

Culture Samples Synechococcus WH7803

Environmental Samples 1L seawater at BATS

Cell dilutions (10^7 to 10^3 cells)

Cell concentrations (From 1L to 5ml)

Cell sorting (~10^6 cells)

Cell lysis

MDA+ Illumina library

Transposon-based library

Illumina Sequencing

Data Analysis

Figure S1: Overview of the steps of the method used for targeted metagenomic sequencing of *Prochlorococcus* and *Synechococcus* for environmental and culture samples.
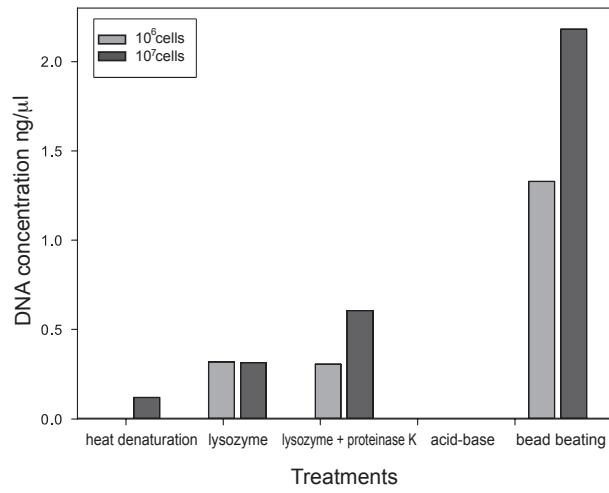
Figure S2: Outcome of different cell lysis treaments (a to e) as a function of DNA concentration in ng/μl on the sorted *Synechococcus* cells with two starting amounts of DNA.
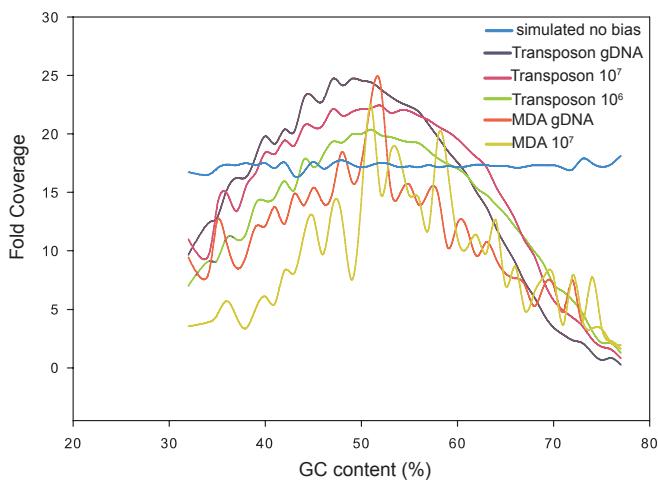
Figure S3: Mean coverage depth for genomic regions defined by GC content was analyzed for different DNA starting amounts and method: transposon-based and MDA. The "no bias" curve represents same size-matched fragments randomely selected (x100) from the reference genome to model non-biased coverage. There was no significant effect of treatment (gDNA vs. 10^7 vs. 10^6) for the transposon based technique ($P > 0.05$) and no difference in bias toward the simulated no bias line ($P > 0.05$). There was a significant effect of treatment (gDNA vs 10^7) and bias toward the simulated no bias line for the MDA technique ($P < 0.05$). There was no significant effect of bias of both gDNA's when compared to the simulated line ($P > 0.05$) but there was significant differences between MDA and transposon-based technique ($P < 0.05$) (Welch's t test). gDNA is equivalent to 2ng of genomic DNA used as input for each method.
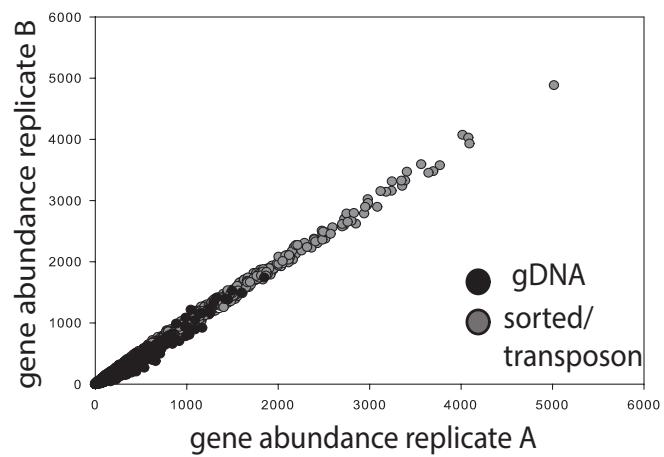
Figure S4: Relationship between the replicated samples for each bulk environmental genomic DNA and sorted *Synechococcus* transposon sample not normalized to the same number of matched sequences.

Table S1: Location, sampling date and corresponding environmental and sequencing values of samples analyzed

Sample	Environment	Position	Date	Depth (m)	%Matches	FCM (10^6)	Method	Cell sorting
B258proS (rep 1)	Atlantic	31 40N 61 10W	May 2010	Surface	11.35	3.0	MDA	*Prochlorococcus*
B258proS (rep 2)	Atlantic	31 40N 61 10W	May 2010	Surface	18.89	2.8	MDA	*Prochlorococcus*
B258pro80 (rep 1)	Atlantic	31 40N 61 10W	May 2010	80m	40.72	2.4	MDA	*Prochlorococcus*
B258pro80 (rep 2)	Atlantic	31 40N 61 10W	May 2010	80m	43.86	2.2	MDA	*Prochlorococcus*
B258synS (rep 1)	Atlantic	31 40N 61 10W	May 2010	Surface	16.06	3.1	MDA	*Synechococcus*
B258synS (rep 2)	Atlantic	31 40N 61 10W	May 2010	Surface	16.98	3.0	MDA	*Synechococcus*
B261proS (rep 1)	Atlantic	31 40N 61 10W	Aug 2010	Surface	41.30	3.6	Transposon	*Prochlorococcus*
B261proS (rep 2)	Atlantic	31 40N 61 10W	Aug 2010	Surface	41.26	3.0	Transposon	*Prochlorococcus*
B261pro80 (rep 1)	Atlantic	31 40N 61 10W	Aug 2010	Surface	66.69	10	Transposon	*Prochlorococcus*
B261pro80 (rep 2)	Atlantic	31 40N 61 10W	Aug 2010	Surface	66.63	10	Transposon	*Prochlorococcus*
B261synS (rep 1)	Atlantic	31 40N 61 10W	Aug 2010	Surface	96.32	1.9	Transposon	*Synechococcus*
B261synS (rep 2)	Atlantic	31 40N 61 10W	Aug 2010	Surface	96.34	1.5	Transposon	*Synechococcus*
B261syn80 (rep 1)	Atlantic	31 40N 61 10W	Aug 2010	80m	92.27	1.0	Transposon	*Synechococcus*
B261syn80 (rep 2)	Atlantic	31 40N 61 10W	Aug 2010	80m	92.31	1.0	Transposon	*Synechococcus*
B270proS	Atlantic	31 40N 61 10W	June 2011	Surface	25.64	1.5	Transposon	*Prochlorococcus*
B270pro80	Atlantic	31 40N 61 10W	June 2011	80m	53.81	2.0	Transposon	*Prochlorococcus*
B270synS	Atlantic	31 40N 61 10W	June 2011	Surface	96.18	1.0	Transposon	*Synechococcus*
B272pro80	Atlantic	31 40N 61 10W	Aug 2011	80m	35.36	2.0	Transposon	*Prochlorococcus*

B272synS	Atlantic	31 40N 61 10W	Aug 2011	Surface	95.36	1.0	Transposon	*Synechococcus*
B262synS	Atlantic	31 40N 61 10W	Sept 2010	Surface	90.25	1.0	Transposon	*Synechococcus*
B263synS	Atlantic	31 40N 61 10W	Oct 2010	Surface	96.06	1.0	Transposon	*Synechococcus*
B264synS	Atlantic	31 40N 61 10W	Nov 2010	Surface	96.19	1.0	Transposon	*Synechococcus*
CA-1A	Pacific	31 40N 61 10W	Feb 2011	Surface	59.57	3.0	Transposon	*Synechococcus*
CA-1B	Pacific	33 22N 117 59W	Feb 2011	Surface	65.08	3.0	Transposon	*Synechococcus*
CA1	Pacific	33 22N 117 59W	Feb 2011	Surface	8.90	-	Tru-Seq	Bulk/no sorting
CA2	Pacific	33 22N 117 59W	Feb 2011	Surface	5.84	-	Tru-Seq	Bulk/no sorting

Table S2: Details of the data set used based on sequencing values (NX is the transposon-based method)

Sample	#Sequences (10^6)	%Matches	%Covered	Coverage
NX-gDNA	1.25	86.03	99.56	27.16
NX-10^7	1.25	58.91	99.12	16.54
NX-10^6	1.25	58.81	99.80	16.45
NX-10^5	1.25	47.76	99.81	13.72
NX-10^4	1.25	14.59	97.79	3.73
NX-10^3	1.25	1.69	28.89	0.43
MDA-gDNA	1.25	47.71	64.14	14.84
MDA-10^7	1.25	14.68	82.34	4.53
MDA-10^6	1.25	11.78	34.90	3.39
MDA-10^5	1.25	5.72	11.07	1.75
MDA-10^4	1.25	4.44	97.19	1.25
MDA-10^3	1.25	0.08	28.78	0.02

Table S3: Summary of phosphate and nitrogen acquisition gene sequences used in this study using *Synechococcus* WH8102 and *Prochlorococcus* MED4 as references. Nitrogen genes in *Prochlorococcus* were generated and not found in genebank.

genes	Genes ID	
	WH8102	Med4
phoB	NP_897040.1	NP_892823.1
phoR	NP_897041.1	NP_892824.1
707		NP_892825.1
phoA		NP_892826.1
phoE	NP_898316.1	NP_892827.1
pstS	NP_897111.1	NP_892828.1
chrA	NP_897416.1	NP_892829.1
mfs	NP_896893.1	NP_892830.1
gap1	NP_896892.1	NP_892831.1
714	NP_896891.1	NP_892832.1
715		NP_892833.1
arsA	NP_897132.1	NP_892834.1
717		NP_892835.1
ptrA	NP_897112.1	NP_892836.1
719	NP_896959.1	NP_892837.1
720		NP_892838.1
721		NP_892839.1
722		NP_892840.1
pstC	NP_897363.1	NP_892841.1
pstA	NP_897364.1	NP_892842.1
pstB	NP_897365.1	NP_892843.1
sphX	NP_897379.1	
AP1	NP_896391.1	
AP2	NP_898479.1	
AP3	NP_898480.1	
psiP1	NP_896260.1	
som	NP_898315.1	
phoX (syn)	NP_897890.1	
phoX-1		NC_999991protein1
phoX-2		NC_999991protein2
nirA	NP_898566.1	NC_999999protein1
moaD	NP_898562.1	NC_999999protein10
narX1	NP_898555.1	NC_999999protein11
nirX	NP_898578.1	NC_999999protein12
narX2	NP_898554.1	NC_999999protein13
narB	NP_898553.1	NC_999999protein2
moaA	NP_898549.1	NC_999999protein3
moaB1	NP_898563.1	NC_999999protein4
moaE	NP_898561.1	NC_999999protein5
mobA	NP_898550.1	NC_999999protein6
moeA	NP_898558.1	NC_999999protein7
napA		NC_999999protein8
napA1	NP_898551.1	
napA2	NP_898552.1	
moaC	NP_898557.1	NC_999999protein9

Table S4: GenBank accession numbers for the metagenomes

Accession	Alias	Description
SRR1029009	B258_pro25-1a	B258 *Prochlorococcus* surface rep 1
SRR1029010	B258_pro25-1b	B258 *Prochlorococcus* surface rep 2
SRR1029011	B258_pro26-1a	B258 *Prochlorococcus* 80m rep 1
SRR1029012	B258_pro26-1b	B258 *Prochlorococcus* 80m rep 1
SRR1029013	B258_syn25-1a	B258 *Synechococcus* surface rep 1
SRR1029014	B258_syn25-1b	B258 *Synechococcus* surface rep 2
SRR1029015	NXca1Aa	California Current *Synechococcus* rep 1
SRR1029016	NXca1Ab	California Current *Synechococcus* rep 2
SRR1029018	CA1A_gDNA	Bulk DNA California Current rep 1
SRR1029019	CA1B_gDNA	Bulk DNA California Current rep 1
SRR1029020	NXproSa	B261 *Prochlorococcus* surface rep 1
SRR1029021	NXproSb	B261 *Prochlorococcus* surface rep 2
SRR1029022	NXpro80a	B261 *Prochlorococcus* 80m rep 1
SRR1029023	NXpro80b	B261 *Prochlorococcus* 80m rep 2
SRR1029024	NXsynSa	B261 *Synechococcus* surface rep 1
SRR1029025	NXsynSb	B261 *Synechococcus* surface rep 2
SRR1029026	NXsyn80a	B261 *Synechococcus* 80 m rep 1
SRR1029028	NXsyn80b	B261 *Synechococcus* 80 m rep 2
SRR1029034	B270proS_CCTGGT_L002_R1	B270 *Prochlorococcus* surface
SRR1029035	B270pro80_TCTTTG_L00	B270 *Prochlorococcus* 80m

Accession	Alias	Description
	2_R1	
SRR1029036	B270synS_AGTTGG_L002_R1	B270 *Synechococcus* surface
SRR1029037	B272synS_GTTATG_L002_R1	B272 *Synechococcus* surface
SRR1029038	B272pro80_CGACTT_L003_R1	B272 *Prochlorococcus* 80m
SRR1029039	B262synS_ACCTTT_L001_R1	B262 *Synechococcus* surface
SRR1029040	B263synS_GACACG_L001_R1	B263 *Synechococcus* surface
SRR1029041	B264synS_ACGTCC_L001_R1	B264 *Synechococcus* surface

Table S5: GenBank accession numbers for the cultured *Synechococcus* WH7803

Accession	Alias	Description
SRR1029108	mdaC3	MDA 10^3 cells
SRR1029109	mdaC4	MDA 10^4 cells
SRR1029110	mdaC5	MDA 10^5 cells
SRR1029111	mdaC6	MDA 10^6 cells
SRR1029112	mdaC7	MDA 10^7 cells
SRR1029114	mdapos1	MDA gDNA
SRR1029115	nxC3	Transposon 10^3 cells
SRR1029133	nxC4	Transposon 10^4 cells

Accession	Alias	Description
SRR1029135	nxC5	Transposon 10^5 cells
SRR1029136	nxC6	Transposon 10^6 cells
SRR1029138	nxC7	Transposon 10^7 cells
SRR1029139	nxpos1	Transposon gDNA