Table 1. Current gene targets and status

GENE	FUNCTION	NOTES	PURPOSE	SPECIFICITY	RELEVANCE	WHERE ARE WE?
PHOSPHORUS						
pstS	phosphate transport: high-affinity phosphate binding protein	prokaryotic; often is present in several copies per genome; not all copies are induced by P limitation	**qPCR**: presence or absence of a genetic potential to scavenge low concentrations of Pi; **qRT-PCR**: activation of pstS expression indicates P limitation;	Cyanobacterial group specific: N2-fixing vs non-fixing or genus specific; Syn groups	Indicate Pi limitation	Have to start by sequencing genes from MB, ALOHA
phoA	utilization of various organic phosphates: alkaline phosphatase	Eukaryotic and prokaryotic; activity is observed under P limitation: eukaryotic - response to external P; cyanobacteria - response to internal P status (phoR is soluble, but an unknown mechanism of activation may exist); single or multicopy gene	**qPCR**: presence or absence of a genetic potential to utilize alternative source of P from DOP; **qRT-PCR**: activation of phoA expression indicates P limitation;	Group-specific (phylum): to differentiate the responses between cya, bacteria, and eukaryotes	Indicates Pi limitation.	Have to start by sequencing genes from MB, ALOHA
phoX	alkaline phosphatase	More abundant than phoA in the marine environment. Four clusters (based on metagenomic surveys and env PCRs): Proteobacteria-only, Roseo-only, 'other' gammas and Bacteriodetes, Cyanos	**qPCR**: presence or absence of a genetic potential to utilize alternative source of P from DOP; **qRT-PCR**: activation of phoX expression indicates P limitation;	gamma-specific, alpha-specific, Syn and Pro-specific	Indicates Pi limitation.	Have to start by sequencing genes from MB, ALOHA
phnD	phosphonate transport: phosphonate-binding protein	in Syn, expression is activated when DIP is low; in Pro HLII clade, expression is constitutive; Syn and Pro phn genes do not belong to the pho regulon; in other cya that have C-P lyase pathway, expression of phnD is regulated by phoBR; in eubacteria, regulation is P dependent or activation by PN presence	**qPCR**: presence or absence of a genetic potential to utilize phosphonates (2-AEP); **qRT-PCR**: activation of phnD expression (in Syn) indicates P limitation or P starvation, but may be a different profile from pstS and phoA expression;	genera specific	Low DIP, utilization of phosphonates	Have to start by sequencing genes from MB, ALOHA;
phnJ (or another conserved gene from phnG-M)	phosphonate utilization by C-P lyase pathway	some cyanobacteria (other than Syn and Pro) and bacteria; single copy	**qPCR**: presence or absence of a genetic potential to utilize (and synthesize?) various phosphonates and produce methane; **qRT-PCR**: activation of phnJ expression indicates P limitation and potential assimilation of phosphonate pool and production of methane	Two groups (if possible): cyanobacterial and bacterial	Low DIP, utilization of phosphonates, CH4 release	Have to start by sequencing genes from MB, ALOHA
IRON						
idiA	iron transport in some bacteria and cyanobacteria	cyanobacterial and bacterial; srl0513=idiA is a periplasmic iron binding protein in Syn PCC 6803 (other names: futA, sfuA, hitA, mapA?); consider also genes: isiA, dpsA, isiB, fur	**qPCR**: presence or absence of a genetic potential to scavenge Fe ; **qRT-PCR**: Fe limitation	cyanobacterial groups: N2-fixing, non-fixing or genera specific; diatoms, SAR11 and other bacteria	**Fe limitation. MB:** Johnson et al. (2001) observe high Fe flux at first Spring upwelling event, followed by [Fe] decrease while upwelling continues. Monitoring *idiA* expression will reveal the time scale of the biological response and whether the decrease is due to increased biological uptake after an adaptation period (could also be abiotic). (Up June to Aug, then decreases through to Oct '99) Relevant at all stations C1, M0, M1 (and ALOHA)	Have to start by sequencing genes from MB, ALOHA
NITROGEN						
nifH	N fixation	more important in oligotrophic areas; diel periodicity (grp A more active in day, grpB at night) depending on O2 production (PS II) or localization (heterocysts)	**qPCR**: presence of N-fixers; **qRT-PCR**: degree to which N is fixed (which in turn influences primary productivity)	gamma proteobacteria, grp A, B, C, tricho and heterocyst-cluster 1, 2, and 3	**MB:** Johnson attempted to measure N2 fixation and it was negligible, even at his "oligotrophic" station off shore along CalCOFI transect line 67. **ALOHA:** relevant and changes over time (Church et al., 2005)	Probes for gammas, grp A, B, C, tricho and heterocyst-cluster 1, 2, and 3 already developed and ordered, just need to optimize with ESP reagents.
urtABE	urea transport	all 3 genes necessary to make the permease; regulated by ntcA; expressed in response to N-limitation	**qPCR**: ability of community to transport urea; **qRT-PCR**: active urea transport (N-stressed population)	Cyanobacteria, diatoms, Proteobacteria	**MB:** Johnson et al. observe uncoupling of primary production max (using O2 as a proxy) and NO3 max, in verticle profiles. Has never looked at organic N sources. Likely to be most significant nearshore (and change with extent of ag runoff. Kudela's group (2007) show increased domoic acid production with urea vs. other N sources.	Have to start by sequencing ureases from MB, ALOHA
ureC	urease	production of NH4, CO2; constitutively expressed in some organisms and may not be a good indicator of urea utilization	**qPCR**: ability of community to use urea (if transported); **qRT-PCR**: constitutively expressed so maybe doesn't mean much	Syn and Pro, diatoms?, Proteobacteria?	same as urtA	Have to start by sequencing ureases from MB, ALOHA
ntcA	global N regulator	regulates expression of several operons involved in utilization of alternate N sources	**qPCR**: Ability to respond to ammonium limitation (and likely use other sources of N); **qRT-PCR**: Ammonium limitation	Syn and Pro	N limitation	Have to start by sequencing ntcA from MB, ALOHA
amoA	ammonia oxidation	ammonia oxidation to NO2 by Crenarchaeota, Bacteria	**qPCR**: presence of ammonia oxidizers; **qRT-PCR**: active oxidation of ammonia	Archaeal, Bacterial	Already done in **MB** and **ALOHA** (Mincer et al., 2007)- Archaeal amoA abundance higher at ALOHA	Primers, probes developed for MB, ALOHA
METHANE						
pmoA??	aerobic methanotrophy	water column supersaturated with methane (w/respect to the atm)	aerobic methanotrophy in the water column	gamma proteobacterial	AOA amoA transcript profiles in Monterey Bay (Santoro in press) might look more like CH4 profiles than NH3 profiles (Tillbrook and Karl, 1999; not the case for AOB). pmoA and amoA are homologues and both can usually act on both methane and ammonia*	Have to start by sequencing genes from MB, ALOHA

Table 2. Current status of biogeochemical cycling probe implementation on ESP

Gene	Organism/group	Assay type	Reference	Location	Has been run on ESP?	Has been deployed?	Benefits	Science
16S rRNA	Crenarchaea	TaqMan	Preston et al., unpubl.	Monterey Bay	Y	Y	ABC	Big players in N cycling in Monterey Bay. Correlation between Cren numbers and [NO3]? Will also let us know which wcrs to target for furth
16S rRNA	SAR11	TaqMan	"	Monterey Bay	Y	Y	ABC	Become more abundant with upwelling
ammonia monooxygenase, grp A	Crenarchaea	TaqMan	Mosier et al., unpubl.	Monterey Bay	Y	Y	D	Subclades of Crens vary in abundances over depth. Might give us another indication of stratification or upwelling..
ammonia monooxygenase, grp B	Crenarchaea	TaqMan	"	Monterey Bay	Y	Y	D	"
ammonium transporters, amt	Synechococcus	TaqMan	Turk et al., unpubl.	Monterey Bay			A	Big players in C, N cycling in Monterey Bay. Will help to characterize what dictates their blooms
assim. nitrate reductase, grp A	Synechococcus	TaqMan	Paerl et al., 2009	Monterey Bay			D	N stress and utilization of NO3. Will help to characterize the conditions under which various subclades dominate
assim. nitrate reductase, grp B	Synechococcus	TaqMan	"	Monterey Bay	Y	Y	D	"
assim. nitrate reductase, grp C	Synechococcus	TaqMan	"	Monterey Bay			D	"
assim. nitrate reductase, grp D	Synechococcus	TaqMan	"	Monterey Bay	Y		D	"
assim. nitrate reductase, grp E	Synechococcus	TaqMan	"	Monterey Bay	Y	Y	D	"
dimethylsulfoniopropionate demethylase	Roseobacter sp.	TaqMan	Varaljay et al., unpubl.	Monterey Bay			ABC	DMS from release and subsequent cloud nucleation
dimethylsulfoniopropionate demethylase, D/1	SAR11	SYBR Green	Varaljay et al., unpubl.	Monterey Bay	Y		ABC	Can tell whether blooms of dmdA-containing SAR11 subclades coincide with phytoplankton blooms
dimethylsulfoniopropionate demethylase, D/1	SAR11	TaqMan	"	Monterey Bay	Y		ABC	"
dimethylsulfoniopropionate demethylase, D/1	SAR11	TaqMan+	"	Monterey Bay	Y		ABC	"
dimethylsulfoniopropionate demethylase, D/3	SAR11	SYBR Green	"	Monterey Bay	Y		ABC	"
dimethylsulfoniopropionate demethylase, D/3	SAR11	TaqMan	"	Monterey Bay	Y		ABC	"
dimethylsulfoniopropionate demethylase, D/3	SAR11	TaqMan+	"	Monterey Bay	Y		ABC	"
DMSP lyase	Roseobacter sp.	TaqMan	"	Monterey Bay			ABC	Production of DMS
nitrogenase	gamma proteobacteria	TaqMan	Church et al., 2005	Hawaii			A	Nitrogen fixation by diferent diazotroph groups
nitrogenase	heterocystous cyano	TaqMan	"	Hawaii			A	"
nitrogenase	unicellular cyano, grp A	TaqMan	"	Hawaii			A	"
nitrogenase	unicellular cyano, grp B	TaqMan	"	Hawaii			A	"
phosphatase, phoX	Synechococcus	TaqMan	Padilla et al., unpubl.	Monterey Bay			A	P limitation, utilization of organic P source
phosphonate lyase, phnJ	"other cyano" (not Syn, Pro)	TaqMan	Cote et al., unpubl.	Hawaii			A	Low DIP, phosphonate utilization and CH4 release
phosphonate transport, phnD	Synechococcus	TaqMan	"	Monterey Bay			A	Low DIP, utilization of phosphonates
RuBisCO	Deep clades	SYBR Green	Preston et al., unpubl.	deep sea	Y	Y		Abundances
RuBisCO	Synechococcus	TaqMan	Turk et al., unpubl.	Monterey Bay	Y	Y	A	Big players in C, N cycling in Monterey Bay. Will help to characterize what dictates their blooms, etc. Also lets us know what groups to target for other genes
urease, cluster B	gamma proteobacteria	TaqMan	"	Monterey Bay			ABC	Urea utilization
urease, cluster C	Roseo-like (alpha)	TaqMan	"	Monterey Bay			C	"
urease, cluster E	Roseo-like (alpha)	TaqMan	"	Monterey Bay			C	"
urease, grp A	Synechococcus	TaqMan	"	Monterey Bay			A	"
urease, grp B	Synechococcus	TaqMan	"	Monterey Bay			A	"

Notes:
A: temporal
B: robust assay
C: Target is always abundant and can be as positive control
D: Paerl et al., submitted; Santoro and Francis

Table 3.Cyanobacterial probes that have been successfully applied to environmental samples

CATEGORY	TARGET	FUNCTION	METHOD	RELEVANCE	SPECIFICITY	REFERENCE	NOTES
CELL CYCLE	ftsZ	cell cycle protein	qRT-PCR	High expression indicates beginning of cell division	Prochlorococcus	Holtzendorff et al., 2002	Diel expression
DIVERSITY	rbcL	ribulose-1,5-bisphosphate carboxylase/oxygenase	PCR/RT-PCR	Diversity and activity of phytoplankton	All phytoplankton	Pichard et al., 1997	Diel expression
DIVERSITY	psbA	Photosystem II core protein D1	PCR	PCR: diversity/abundance of photosynthetic organisms	Photosynthetic organisms (eukaryotes and cyanobacteria)	Zeidner et al., 2003	Diel expression
DIVERSITY	16S-23S ITS	intergenic spacer between 16S and 23S genes	PCR	PCR: diversity/abundance of cyanobacteria	Cyanobacteria	Rocap et al., 2002	
DIVERSITY	rpoC1	DNA-dependent RNA Polymerase	PCR	PCR: diversity/abundance of cyanobacteria	Cyanobacteria and Prochlorophytes	Palenik and Hazelkorn, 1992	
DIVERSITY	cpcBA-IGS	region of cpc (phycocyanin) including intergenic spacer and cpcA, cpcB flanking regions	PCR	PCR: Synechococcus diversity and phycobilin content	Synechococcus spp.	Robertson et al., 2001	
DIVERSITY	cpeB	phycoerythrin	PCR	PCR: Prochlorococcus HL and LL diversity/ abundance	Prochlorococcus Hl and LL	Steglich et al., 2003	
DIVERSITY	16S rRNA	16S ribosomal RNA gene	PCR	diversity	1. bacteria and archaea; 2. Groups: cyanobacteria, alpha, beta, gamma-proteobacteria, bacteroidetes, planctomycetes, firmicutes	1. Weisburg et al., 1991; 2. Ashelford et al., 2002; Muhling et al., 2008	For group-specific, used DGGE PCR
IRON	isiB	flavodoxin	qRT-PCR	Expression indicates iron limitation	two Trichodesmium clades	Chappell and Webb, 2009	
IRON	isiA	iron stress induced protein	qRT-PCR (TaqMan)	Iron and/or oxidative stress.	Crocosphaera	Hewson et al., 2009	
NITROGEN	nifH	dinitrogen reductase	PCR, qPCR, qRT-PCR(TaqMan)	1. qPCR: diversity and abundance of nitrogen fixing organisms; 2. qRT-PCR: their participation in nitrogen fixation	1. Degenerate for all; 2. Cyanobacterial; 3. Group-specific: Gr. A, B, III, Trichodesmium	1.Zehr and McReynolds, 1989; Zani et al., 2000; 2. Olson et al., 1999; 3. Church et al., 2005	Diel expression
NITROGEN	ntcA	nitrogen global control transcription regulator	PCR/RT-PCR	1. PCR: diversity/ abundance;2) RT-PCR: Expression shows nitrogen (NH4+) limitation	1. Picocyanobacteria 2. Synechococcus-specific	Lindell and Post, 2001; Penno et al., 2006	Expression shows nitrogen (NH4+) limitation (Lindell et al., 2005)
NITROGEN	cynA	cyanate-binding protein, ABC transporter subunit	PCR	PCR: a genetic ability to acquire cyanate from outside	1. Cyanobacteria; 2. Synechococcus; 3. Prochlorococcus	Kamennaya et al., 2008	Expression in N-limited cultures (Tolonene et al., 2006)
NITROGEN	urtA	urea-binding protein, ABC transporter subunit	PCR	PCR: urea acquisition	1. Cyanobacteria; 2. Synechococcus	Kamennaya et al., 2008	
NITROGEN	nifD	dinitrogenase	PCR	diversity	cyanobacteria	Roeselers et al., 2007	
NITROGEN	narB	nitrate reductase	PCR	PCR: an ability to assimilate nitrate	Cyanobacteria	Jenkins et al., 2006	
PHOSPHORUS	pstS (sphX)	high affinity phosphate binding protein, ABC transporter	qRT-PCR	Expression indicates Pi limitation	Trichodesmium	Orchard et al., 2009	multiply diverse copies
PHOSPHORUS	phoA	phosphatase (Zn2+)	qRT-PCR	Expression indicates Pi limitation	Trichodesmium	Orchard et al., 2009	
PHOSPHORUS	phoX	phosphatase (Ca2+)	1.PCR; 2.qRT-PCR	1. PCR: Presence of PhoX type (Ca2+) phosphatase; 2. Expression indicates Pi limitation and utilization of organic phosphates	1. Bacteria; 2. Trichodesmium	1. Sebastian and Amermman, 2009; 2. Orchard et al., 2009	
PHOSPHORUS	phnD	phosphonate-binding protein; ABC transporter	RT-PCR	Expression indicates Pi limitation and potential utilization (transport) of phosphonates	1. Trichodesmium; 2. Synechococcus-specific, Prochlorococcus-specific	1. Dyhrman et al., 2006; 2. Ilikchyan et al., 2009	In some cya (e.g. Trichodesmium), multiply diverse copies of phnD
PHOSPHORUS	phnJ	C-P lyase protein	PCR/RT-PCR	1. Presence of C-P lyase pathway for phosphonate utilization; 2. Expression indicates Pi limitation and potential phosphonate utilization	Trichodesmium	Dyhrman et al., 2006	
TOXIN	ndaF	nodularin synthetase gene subunit F	qPCR	Quantification of toxin-producing Nodularia	Nodularia	Koskenniemi et al., 2007	Specificity: 30 copies per L
BIOSYNTHESIS	nrps	nonribosomal peptide synthetases	PCR	A genetic ability to synthesize natural products	Cyanobacteria	Ehrenreich et al., 2005	
BIOSYNTHESIS	mps	modular polyketide synthases	PCR	A genetic ability to synthesize natural products	Cyanobacteria	Ehrenreich et al., 2005	