

3-4-2004

## Detection And Quantification Of Karenia Brevis By Carbon Fixation Gene Expression Analysis

Michael Alan, Gray  
*University of South Florida*

Follow this and additional works at: <https://digitalcommons.usf.edu/etd>



Part of the [American Studies Commons](#)

---

### Scholar Commons Citation

Gray, Michael Alan,, "Detection And Quantification Of Karenia Brevis By Carbon Fixation Gene Expression Analysis" (2004). *USF Tampa Graduate Theses and Dissertations*.  
<https://digitalcommons.usf.edu/etd/1053>

This Thesis is brought to you for free and open access by the USF Graduate Theses and Dissertations at Digital Commons @ University of South Florida. It has been accepted for inclusion in USF Tampa Graduate Theses and Dissertations by an authorized administrator of Digital Commons @ University of South Florida. For more information, please contact [digitalcommons@usf.edu](mailto:digitalcommons@usf.edu).

Detection And Quantification Of Karenia Brevis By Carbon Fixation Gene Expression  
Analysis

by

Michael Alan Gray

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science  
College of Marine Science  
University of South Florida

Major Professor: John H. Paul, Ph.D.  
Gabriel Vargo, Ph.D  
David Mann, Ph.D

Date of Approval:  
March 4, 2004

Keywords: rbcl, real-time pcr, red tide, harmful algal bloom, monitoring, gulf of mexico,  
gymnodinium breve

© Copyright 2004 , Michael A. Gray

## Table of Contents

List of Tables	iii
List of Figures	iv
Abstract	vii
Chapter One: Introduction	1
Chapter Two: Development of assay, sensitivity and specificity testing	16
Introduction	16
Methods	18
Design of Real-Time RT-PCR Primer and Probe Set	18
Culture Conditions	18
DNA extraction, amplification, cloning and sequencing	19
Sequence analysis	20
Testing of the Real Time RT-PCR Primer and Probe Set	21
Optimization of Reaction Conditions	21
Sensitivity and specificity testing	21
Results	23
Phylogenetic analysis	23
Real-time RT-PCR	30
Discussion	41
Chapter Three: Testing of the assay, field samples, diel studies and method evaluation	43
Introduction	43
Methods	45
RNA extraction and amplification from environmental samples	45
Diel Experiments	46
Cultures and culture conditions	46
Sampling schedule, cell counts and RNA extraction	46
Carbon Fixation	47
Alkalinity determination	48
Evaluation of real-time assay reagents and extraction methods	48
Room temperature and freezer (-80° C) stability of <i>K. brevis</i> lysates	48

Alternative extraction methods	49
Results	51
Field samples	51
Diel studies	56
Room temperature stability in lysis buffer	63
Freezer stability in lysis buffer	64
Alternative extraction methods	64
Discussion	74
References	83
Appendices	90

## List of Tables

Table 1: Percent identity of <i>rbcL</i> sequences from selected algal species	25
Table 2: Accession for sequences used in phylogenetic analyses	28
Table 3: Positive and negative controls for amplification by real-time RT-PCR	31
Table 4: Bloom samples counted by microscopy and inferred counts by real-time RT-PCR	53

## List of Figures

Figure 1: Theoretical amplification curves that would be generated through real-time RT-PCR	11
Figure 2: The Taq nuclease assay (TNA) process	13
Figure 3: Phylogenetic tree based on <i>rbcL</i> amino acid sequences	27
Figure 4: An example standard curve generated using in vitro transcript	34
Figure 5: An example standard curve generated using whole cell RNA extract	36
Figure 6: Composite of all transcript standard curves	38
Figure 7: Composite of all cell standard curves	40
Figure 8: Cell counts by microscopy the real-time RT-PCR assay	55
Figure 9: Carbon fixation over the diel periods	58
Figure 10: RNA transcription over the diel periods	60
Figure 11: Average carbon fixation and average <i>rbcL</i> transcription for all of the diel experiments	62
Figure 12: RNA stability at room temperature	
A) Room temperature stability in Qiagen's RLT buffer	66
B) Room temperature in Stratagene's Lysis buffer	67
Figure 13: Average percent of initial RNA signal after storage at room temperature	69
Figure 14: RNA stability in Stratagene's Lysis buffer after storage at $-80^{\circ}\text{C}$	71
Figure 15: Detected <i>rbcL</i> RNA signal following alternative extraction methods	73

Figure 16: Cell counts by microscopy and the real-time assay

A) Cell counts by microscopy and the real-time RT-PCR assay not including samples stored at  $-80^{\circ}\text{C}$  for over one month 79

B) Cell counts by microscopy and the real-time RT-PCR assay including samples stored at  $-80^{\circ}\text{C}$  for over one month 80

Detection and Quantification of *Karenia brevis* by Carbon Fixation Gene  
Expression Analysis

Michael Gray

**ABSTRACT**

*Karenia brevis* (Davis cf. Hansen & Moestrup = *Gymnodinium breve*) is the non-peridinin containing dinoflagellate responsible for many harmful algal blooms (red tides) in the Gulf of Mexico. These recurrent blooms can have significant negative ecological, economic, and human health impacts including fish kills, tainting of shellfish, poisoning of marine mammals, loss of tourism revenue due to beach closures, and respiratory distress and food poisoning in humans.

A method for detection of *Karenia brevis* was developed based upon amplification of the mRNA for the plastid-encoded gene of the carbon fixing enzyme ribulose 1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) large subunit (*rbcL*). Using sequence information from a primer set targeting a 554-bp region of the *Karenia rbcL* gene, a small (91 bp amplicon) primer and probe set was created for TaqMan<sup>®</sup> real time RT-PCR of *K. brevis rbcL*. The primer/probe set is sensitive to as little as 0.1 fg of target transcript and as little as 1 pg of total cellular *K. brevis* RNA extract,



corresponding to less than 1 cell reaction<sup>-1</sup>. The primer/probe set did not amplify *rbcL* transcript from any of the non-target algae tested.

Bloom samples analyzed by this method have shown the assay to be a reliable method, with effective enumeration and a linear relationship showing good correlation to the cell counts by microscopy ( $r^2 = 0.8344$ ). The assay has been shown to be robust and perform well even in non-ideal conditions, with pre-extraction RNA from unialgal culture stable at room temperature for up to 3 days and up to a month at  $-80^\circ\text{C}$  in Stratagene's lysis buffer.

The transcription of the *rbcL* gene demonstrated minor variation throughout the diel period, however the variation was not linked to the diel cycle or to carbon fixation, which showed a distinct diel signal. Due to the relatively constant expression of the *rbcL* gene, the real-time RT-PCR assay developed should be able to reliably enumerate *K. brevis* populations in the natural environment, as long as the sample is placed in Stratagene's lysis buffer and processed within one or two days or frozen at  $-80^\circ\text{C}$  and processed within a month.

## **Chapter 1: Introduction**

“... all the waters that were in the river were turned to blood. And the fish that were in the river died; and the river stank, and the Egyptians could not drink of the water of the river” (Exodus 7: 20-21)

Throughout the world, harmful algal blooms (HABs) are chronic and often costly problems that have been a nuisance throughout history. The above quote from the Bible is believed to be the earliest written record of a HAB (22); however coastal communities from much earlier times likely encountered occasional bloom events. The Hebrew dietary rules include an injunction against consuming shellfish, perhaps partly due to their potential to carry HAB contamination. Dale and Yentsch (9) and Carson (6) note that early explorers of North America frequently found Native American tribes with taboos against eating shellfish, as well as some tribes that kept a nightly watch for bioluminescence and daily a watch for discolored water (which can be indicators of a dinoflagellate bloom) in order to avoid harvesting and consuming potentially toxic seafood.

In the Gulf of Mexico, waters stained red, presumably by algal blooms, have been described as early as in the ship’s logs of the Spanish explorers. A massive local bloom was brought to the notice of Gunther (20) who described fish kills from north of the Dry Tortugas as far north as Boca Grande. It was estimated that as many as 50 million fish

including a Goliath Grouper were killed as well as turtles. A patch of water stained yellow, once investigated revealed a large population of *Gymnodinium* sp. (*Karenia* sp.) along with an acrid gas that escaped from the water when boiled, later to be identified as aerosolized brevetoxin. This species was initially described in detail by Davis (10) as an unarmored dinoflagellate, with a width and height ranging from 25 to 32  $\mu\text{m}$ , a thickness of about 12  $\mu\text{m}$ , containing many plastids and a nucleus placed below the girdle and slightly to the left of the sulcus and named *Gymnodinium brevis*. The name has been changed over the years to more closely reflect evolutionary descent community of the group, with the currently accepted name of *Karenia brevis* in recognition of Karen Steidinger's significant contribution to the knowledge about this organism.

The characterization of *K. brevis* has been further expanded to its unusual pigment characteristics. *Karenia brevis* does not contain peridinin, the accessory pigment found in the plastids of most autotrophic dinoflagellates. The primary chemotaxonomic pigments are fucoxanthin, 19'-hexanoyloxyfucoxanthin and 19'-butanyloxyfucoxanthin with smaller concentrations of the accessory pigments  $\beta$ ,  $\epsilon$ -carotene, 19'-hexanoyloxyparacentrone 3-acetate and gyroxanthin diester. The pigment complement found in *K. brevis*, *K. mikimotoi* and *Karlodinium micrum* are very similar and almost identical to the Haptophyte *Pelagomonas calceolata* (4). The unusual plastid pigment complement is due to the incorporation of a plastid from a Haptophyte through tertiary endosymbiosis (30), which it shares with other closely related dinoflagellates, such as *K. mikimotoi* (59). When blooming, *K. brevis* tends to aggregate near the surface of the water (54) and this has been attributed to a combination of positive

phototaxis and negative geotaxis (31), with non-directional dispersal at night. *Karenia brevis* is not constrained to the surface of the water and unless there is a significant pycnocline through which it cannot swim, it has been found throughout the shallow water column. *Karenia brevis* is capable of swimming at a speed of ca. 1 m h<sup>-1</sup> (55).

Blooms of *K. brevis* in the Gulf of Mexico that typically affect the west Florida shelf initiate in the mid-shelf waters (61). Blooms of this organism are not thought to be due to an increased growth rate, but rather the concentration of the organisms through physical processes, thus the term bloom refers to the aggregation of cells, not to accelerated growth (61). Shoreward movement of water masses associated with the Loop Current can carry early bloom populations of *K. brevis* into nearshore waters where they may remain and become more dense, affecting the residents of the nearshore waters. Blooms may move into local estuaries as the water masses in which they are entrained are moved by wind and currents (38).

Throughout the past century, *K. brevis* blooms have been recurrent problems, especially for the west coast of Florida. A chronology of *K. brevis* HAB events compiled by Mote Marine Lab indicates blooms occurring in 30 of the last 57 years and every year since 1991 (14 of 14 years). One of the events in 1952 was so severe that Clearwater Mayor Herbert Brown sought approval to fire-bomb the dead fish with napalm to prevent their arrival onshore. More than 150 tons of dead fish killed by a bloom washed ashore and had to be removed in 1967. While the most often affected animals are fish, there are occasionally other animals affected, including waterfowl (Cormorants (34), Mergansers

and lesser Scaups (12)), Dolphins (15), Manatees, (7 in 1963, (36), 39 in 1982, (46), 149 in 1996, (5), and 98 in 2003 (FMRI press release)) and Sea Turtles.

The effects of *K. brevis* on humans are dependent on the type of exposure. Most commonly this exposure occurs as a result of inhalation of aerosolized toxin, which can lead to irritation of the eyes, nose and throat, and is most likely at or near the beach/shore with an accompanying onshore breeze (48). Less frequent, but more troublesome are cases of ingestion via contaminated seafood. Filter-feeding seafood such as mussels, clams, and oysters can potentially concentrate brevetoxins to levels that are dangerous to humans. Intoxication from contaminated shellfish can result in neurotoxic effects caused by brevetoxin's action on voltage sensitive sodium channels resulting in repeated uncontrolled depolarization of the cell (63). Symptoms can include chills, headache, diarrhea, weakness, muscle and joint pain, paraesthesia, trouble breathing, double vision, and trouble talking or swallowing.

While it is difficult to determine the economic impact of HABs on a yearly basis for the United States of America, Hoagland et al. (27) estimated that the cost is in the range of \$24 to \$83 million per year, including treatment of illnesses, monitoring by state agencies, and tourism and fisheries losses. In the review, Hoagland et al. estimated an annual cost of \$184,000 for monitoring and management in the state of Florida alone based on data from 1987 to 1992.

As *K. brevis* can have such dramatic impacts on human health and the environment and economies of Florida, nearby regions of the Gulf of Mexico and the southern Atlantic coast of the United States, it is clearly important to be able to detect and

quantify these organisms or their toxins from environmental or shellfish samples. The most conventional method to detect and enumerate *K. brevis* from the marine environment is counting by direct microscopic observation. This technique is, unfortunately, tedious, slow and while not technically difficult, requires significant expertise in order to correctly identify *K. brevis* and not count other, less problematic species that may have similar appearances, such as *K. mikimotoi*. *Karenia brevis* identification is further complicated by natural variation of cell size and shape that has been documented at least in culture (69). Even among scientists familiar with identification of dinoflagellates, identification and enumeration by microscopy can include errors. In experiments with pictures of *Dinophysis* species, only 84 to 95% accuracy was returned by experts routinely involved in certain identification tasks (8). Newer direct optical detection techniques have been developed based on flow cytometry and digital imaging technologies and systems have been designed to automatically detect and enumerate certain cell types continuously in land-deployed situations (52).

As the most important impact of *K. brevis* blooms, from a human health perspective, is the contamination of shellfish, there are many methods aimed at detection of brevetoxins in food, water and air samples. The earliest techniques involve detection of toxicity only, using a mouse bioassay. Essentially, a mouse is injected with a sample and toxicity is determined through observation of symptoms and time to death of the mouse. More advanced techniques have been developed involving chromatographic techniques (e.g. HPLC). Other techniques involve antibody immunoassays (for example, (3)) and have been extended to ELISA assays (41) for detection of brevetoxins in various

sample matrices (seawater, shellfish and mammalian body fluid). Concentrations of brevetoxins in marine derived aerosols have been studied using high volume air samplers and HPLC (48).

In addition to the detection of HAB organisms or their effects through toxin analysis and microscopy, methods have been developed based on photopigments to determine the presence and relative abundance of HAB species. Gyroxanthin-diester, relatively rare and very minor photopigment found in *K. brevis*, haptophytes and other *Karenia* species, was targeted for an HPLC-based pigment detection approach (45). Chlorophyll anomalies in near shore waters as detected by satellites have also been used as an indication of HABs (56).

In addition to methods designed to detect the toxins and pigments associated with HABs, there have been developments aimed at the detection of the HAB organisms based on their genetic sequences. One method that could increase the ability of the researcher to correctly identify HAB species (*Heterosigma akashiwo* and *Fibrocapsa japonica*) uses a combination of microscopy and fluorescence in-situ hybridization (FISH) with oligonucleotide probes targeting ribosomal RNA (rRNA) (64). Tyrrell et al. (64) also developed a microtiter plate-based sandwich hybridization assay as an extension of their FISH study, to eliminate the need for microscopy.

There have been several assays designed around amplification and detection of specific sequences such as that of Guillou et al. (19) who targeted the rRNA large subunit for a semi-nested PCR detection method for *Alexandrium*, *Dinophysis* and *Karenia*. This approach has several major drawbacks for the enumeration of HAB species, due to the

non-quantitative nature of endpoint PCR analysis. Godhe (17) also used an approach targeting the SSU rRNA of *Gymnodinium mikimotoi* (recently reclassified as *Karenia mikimotoi*) and the *Alexandrium minutum* species-group using PCR, although, as with the assay of Guillou et al. it was not a quantitative assay; rather, it indicated presence or absence only.

While endpoint analysis of PCR is inherently non-quantitative, it is possible to use a PCR-based assay to determine the concentration of target nucleic acid that was originally in the sample. As the sample progresses through the PCR amplification process, the target sequence accumulates and eventually begins a logarithmic amplification. The time of entry into logarithmic amplification corresponds to the initial concentration of the target sequence. If this entry into logarithmic amplification can be detected, the PCR based assay can be quantitative. There are several approaches to this type of assay. The least technologically demanding method to accomplish quantitative PCR is to use a time-step process. Zhang and Lin (71) developed a time-step PCR procedure to detect *Pfiesteria piscicida* by targeting the mitochondrial cytochrome b gene. In time-step PCR, as defined in the study, 5  $\mu$ l of the PCR reaction was removed at intervals throughout the cycling (at 25 and 30 cycles) and after all 35 cycles had been completed, the reactions were visualized using agarose gel electrophoresis. From this information, approximate cell concentrations in the original sample could be calculated based on the dilution curve generated. This method was able to detect between 0.2 and 15,625 cells per reaction, though saturation of the signal (optical density of the band on the gel) was a significant problem and required dilution of samples or reduction of the



number of amplification cycles. While this method has the potential to be quantitative, it remains a labor-intensive method, requiring careful electrophoresis and analyses at each of the time points in order to accurately and precisely quantify *P. piscicida*.

One technological step further than time-step PCR is “real-time” PCR using fluorescent dye such as SYBR green, which becomes highly fluorescent when bound to DNA. Amplification can then be detected as the reaction is progressing; as more DNA is produced, more SYBR green binds to the DNA, resulting in an increase in fluorescence. This approach was used by many investigators to detect and enumerate a wide variety of organisms and can be a reliably quantitative method.

Another approach to quantitative, real-time PCR is to introduce a third oligonucleotide to the reaction. This oligo serves as a probe and binds to target sequences between the two primers. To this probe are bound two fluorochromes, a reporter and a quencher. When both are bound to the probe, fluorescence is quenched through resonant energy transfer. Through the use of a Taq polymerase with 5’-3’ exonuclease activity (which degrades the bound probe as it amplifies the target sequence) the reporter fluor is released into solution and can fluoresce upon excitation (23). This method, dubbed a Taq nuclease assay (TNA, marketed by Applied Biosystems as TaqMan® PCR) has the potential to be quantitative by detection of the time of a sample’s entry into log phase PCR amplification (which is directly proportional to the initial concentration of the target). Figure 1 shows an example of the amplification curves that are generated in the course of a real-time RT-PCR assay with the time of a samples entry into log phase amplification through a threshold level indicative of the initial

concentration of the target RNA. This method also has the potential to be more specific than standard PCR, due to the introduction of a third oligonucleotide that must also match in sequence the target. See Figure 2 for a graphical representation of the TNA process.

Similar to the assay of Godhe et al., Tengs et al. (60) developed a PCR based assay to detect plastid small subunit rRNA from *Gymnodinium galatheanum*. The critical difference in this assay is that it has the potential to be quantitative, as Tengs et al. used a Taq Nuclease assay.

The study presented here represents a molecular detection and quantitation method for the Florida HAB organism *K. brevis*. The study is presented in two chapters, the first is a description of the development of the real time RT-PCR assay, with sensitivity and specificity testing. The second chapter describes expanded testing of the assay as applied to natural bloom samples, field applicability and robustness as well as testing of the diel variability of *rbcL* gene expression in *K. brevis*.

Figure 1: Theoretical amplification curves that would be generated through real-time RT-PCR. Shown are curves representing ten-fold dilutions of target between each of the samples (solid curves) and the threshold level that indicates a sample's entry into log phase amplification (dashed line).

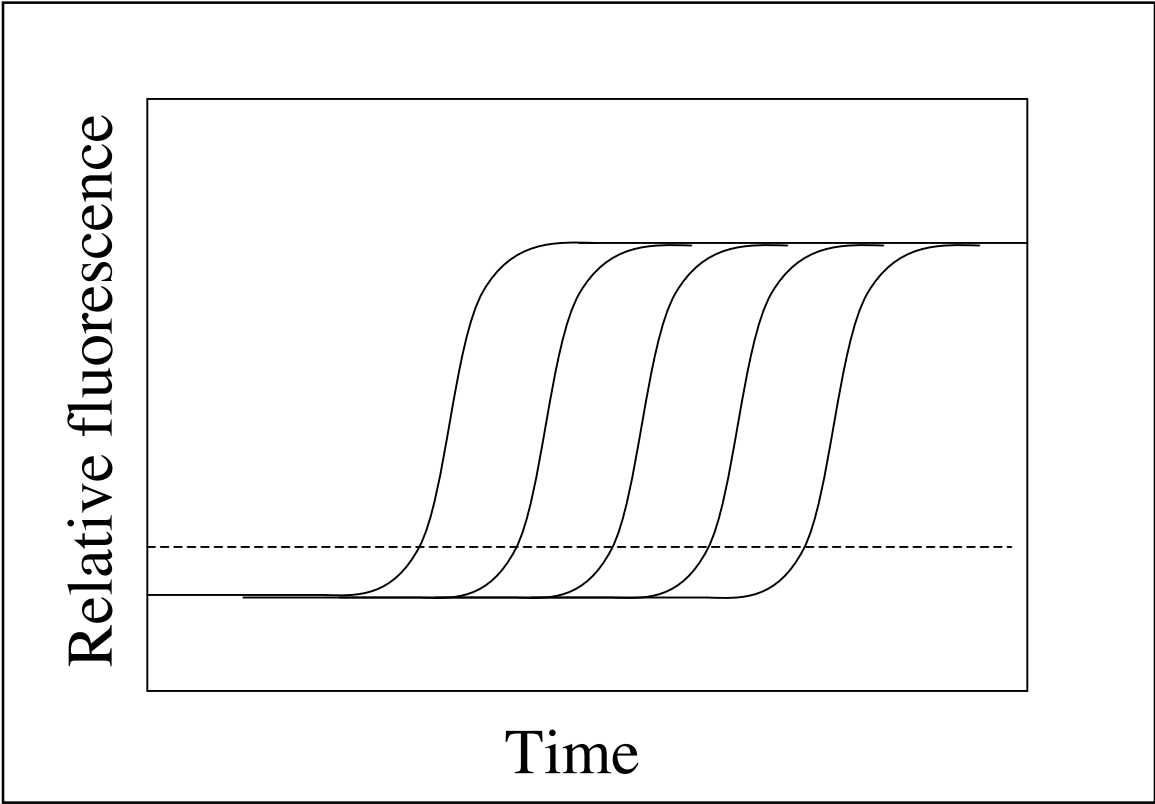
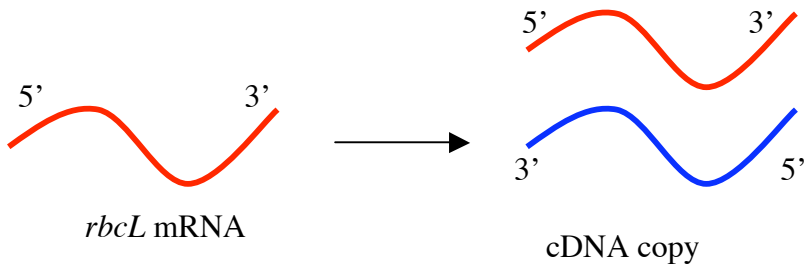


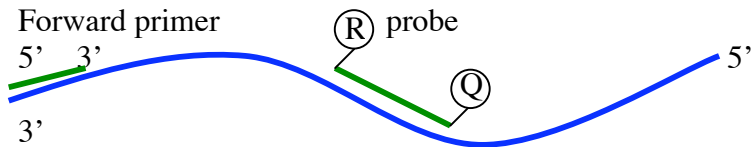
Figure 2: The Taq nuclease assay (TNA) process.

### Step 1, reverse transcription

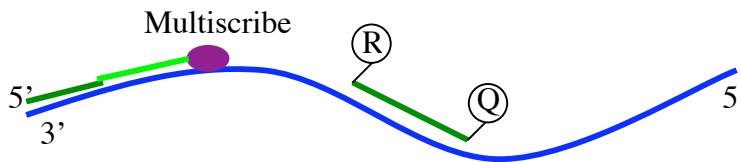


Random hexamers are used as primers for reverse transcriptase or Applied Biosystems' Multiscribe<sup>®</sup> which makes a cDNA copy of all RNA in the reaction. The RT step takes place at 45° C for 30 minutes, followed by initial denaturation at 95° C for 10 minutes.

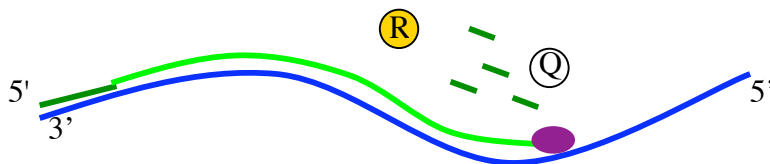
### Step 2, amplification



The forward primer and probe (with its reporter (R) and quencher (Q)) bind to the target sequence. This occurs at 52° C for one minute.



Amplification begins at the forward primer site where Taq DNA polymerase or Multiscribe<sup>®</sup> binds and begins synthesis of new DNA. This occurs at 72° C for one minute.



Amplification continues and as the Multiscribe<sup>®</sup> encounters the probe, the probe is degraded by the enzyme's 5', 3' exonuclease activity. This releases the reporter from the quencher, allowing it to fluoresce and report amplification. After the extension step, the cycle begins again with a 95° C denaturation for one minute.

The detection method chosen for this assay is real time RT-PCR using Applied Biosystems' TaqMan® technology. TaqMan® technology has been demonstrated to be a very reliable and quantitative detection system in a wide range of applications: food microbiology and safety (2, 26), environmental monitoring (21, 43), marine ecosystem research (65), biodefence and clinical pathogen detection (7, 11, 13, 28, 51), cancer research (29, 70), genetic research (50) and others.

In order to have an assay that is sensitive only to viable cells, we targeted mRNA as RNA is rapidly degraded in the environment. The rapid degradation of the RNA in the environment ensures that, if mRNA is detected, it has likely recently been transcribed and therefore the population detected is viable. DNA is a much more stable molecule and it is possible that detection of a DNA target does not reflect the viability of the population detected.

The ribulose 1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) large subunit gene (*rubL*) was chosen as the target of this assay for several reasons. RuBisCO catalyzes the rate-limiting step of photosynthetic carbon fixation and it is notoriously inefficient and slow, therefore a large quantity of enzyme must be made, so the mRNA should be abundant. In addition to catalyzing the addition of CO<sub>2</sub> to ribulose 1, 5-bisphosphate, it can catalyze the addition of O<sub>2</sub> in a competing oxygenase reaction. As RuBisCO is an inefficient enzyme, the cell must maintain a sufficient pool of enzyme to survive, consequently, in land plants, as much as 50% of the nitrogen in leaves is devoted to RuBisCO (see review (53)). Therefore the cell must transcribe many copies of *rubL* in

order to maintain the RuBisCO pool. This high transcription rate will help to greatly increase the sensitivity of the assay as the *rbcL* mRNA will be in significantly higher concentrations than the DNA of the gene. The *rbcL* gene is encoded in the DNA of the plastid, of which *K. brevis* usually has many, further increasing the transcript levels in the cell and thus our limit of detection. Again due to the plastid location of the gene it is likely that *K. brevis* will have an *rbcL* gene sequence that is divergent from that of other dinoflagellates due to the unusual nature of the Haptophyte-derived plastid that *K. brevis* shares with very few other dinoflagellates. As *rbcL* is a functional gene, its evolution may be tightly constrained, however there may be portions of the gene that have more freedom to change in the second or third codon positions without yielding a non-functional enzyme. This high transcription rate, the potential uniqueness, the likelihood that it will not vary within the species due to the relatively recent acquisition of the Haptophyte-derived plastid and the possibility of variable regions within this gene combine to make *K. brevis*' *rbcL* a promising target for a molecular detection and quantitation assay.



## Chapter 2: Development of assay, sensitivity and specificity testing

### Introduction

*Karenia brevis* (Davis cf. Hansen & Moestrup = *Gymnodinium breve*) is an unarmored, non-peridinin containing dinoflagellate that grows to ca. 20-40  $\mu\text{m}$  in diameter. The organism is positively phototactic, negatively geotactic (24, 32), swims at a speed of ca. 1  $\text{mh}^{-1}$  (55) and is thought to be an obligate photoautotroph (1). *Karenia brevis* is the causative agent of the recurring red tide blooms (21 of 22 years from 1975-1997) observed in the Gulf of Mexico and Southeastern Atlantic Coast of the United States (61), which have been reported since the Spanish conquests (20). Lipophilic brevetoxins (37) produced by *K. brevis* can result in massive fish kills and have been implicated in the mortality of 700 bottlenose dolphins off the east coast of the U.S.A. in 1987 (25) and the mysterious deaths of 149 Florida manatees in 1995 and 1996 (63). In human exposure cases, brevetoxin can cause respiratory distress by inhalation and food poisoning by consumption of tainted shellfish.

Current methods for the detection of *K. brevis* depend on microscopy or pigment analysis that are time consuming, and require a considerable amount of expertise and skill (38). Consequently, rapid molecular methods to detect *K. brevis* in the environment are needed. To this end, we have been investigating the ribulose-1, 5-bisphosphate

carboxylase/oxygenase large subunit gene (*rbcL*) as a potential molecular marker for this organism.

The molecular phylogeny of the *rbcL* gene in dinoflagellates is complex. Almost all eukaryotic oxygenic phototrophs contain a functional RuBisCO enzyme composed of both large and small subunits ( $L_8S_8$ ), known as Form I (33) (57)). A second, evolutionarily distant lineage of the enzyme (Form II) composed of large subunits only ( $L_2$ ) is found in certain form I-containing proteobacteria (i.e. *Rhodobacter* and *Rhodospseudomonas*; (16) (58) and interestingly in some dinoflagellates (*Amphidinium carterae*, *Symbiodinium* sp.; (40)). *Karenia brevis* and the other fucoxanthin containing dinoflagellates have a Form ID *rbcL* enzyme and genetic evidence suggests that they contain plastids of Haptophyte origin acquired through tertiary endosymbiosis (30) (59). Sequence information for the *rbcL* genes of many of the dinoflagellates is unavailable, however, due to the apparent ability of dinoflagellates to acquire plastids through retention of the plastids of their prey or some other endosymbiosis mechanism, it is unlikely that further sequencing of *rbcL* genes will elucidate the phylogenetic relationships of Dinophyceae. Evolutionary constraints on the functionality of RuBisCO (giving rise to little sequence variability) also will most likely yield little resolution of phylogenetic relationships. It would be interesting to sequence *rbcL* from many other groups of dinoflagellates in order to possibly determine the ancestral trophic strategies of the dinoflagellates. The presence of a form II RuBisCO in many dinoflagellates (*Amphidinium carterae*, and *Symbiodinium* sp., (68), and *Gonyaulax polyedra*, (40)) is interesting, especially considering that all of the species above contain chloroplasts or

kleptochloroplasts. These chloroplasts may still contain a form I RuBisCO, though it is common for plastids, even if relatively recently acquired, to drastically reduce their genome to the bare minimum for functionality in their new intracellular environment (39). The function of a nuclear encoded, possibly redundant RuBisCO would be interesting to further explore, but is beyond the scope of this study.

As a functional gene, *rbcL* is highly expressed in viable cells. Therefore we targeted the mRNA, which can be orders of magnitude higher in concentration than the DNA of the gene, in order to increase the sensitivity of our assay. As RNA is rapidly degraded in the environment, an RNA target will give an indication of a viable population, compared to DNA based methods, which would detect dead cells as well.

## **Methods**

### Design of Real-Time RT-PCR Primer and Probe Set

### Culture Conditions

Cultures of *K. brevis* were provided courtesy of Karen Steidinger of the Florida Fish and Wildlife Conservation Commission's Florida Marine Research Institute (FMRI). Strains were isolated by her lab from the following locations around the Florida coast: Apalachicola, Charlotte Harbor, Mexico Beach, Jacksonville, and Piney Island. Strains used in this analysis were named for their isolation location and the plate well into which they were isolated. Several non-target algal strains of diverse lineage were obtained from either the Provasoli-Guillard Center for Culture of Marine Phytoplankton (West

Boothbay Harbor, ME) or from the Steidinger lab (see Table 1). All strains were under a 12/12 light regime at  $26 \mu\text{mol s}^{-1} \text{m}^{-2}$  and were incubated at  $20^\circ \text{C}$  or  $14^\circ \text{C}$  in F/2 media (18), modified for each strain's needs, according to CCMP's directions (media recipes and the requirements of each of the tested strains can be found in Appendix 1).

#### DNA Extraction, Amplification, Cloning and Sequencing

*Karenia brevis* cells were harvested by centrifugation (10 minutes at 5000 x G) and the DNA was extracted using a modified phenol/chloroform method (14). A previously designed PCR primer set that amplifies a 554 base pair region of *Karenia*'s *rbcL* gene was used to amplify the gene fragment from *K. brevis*. (Forward primer: GATGATGARAAAYATTA ACTC; reverse primer: ATTTGTCCCGCATTGATTCCT. International Union for Pure and Applied Chemistry degeneracy symbols used.) Polymerase chain reaction (PCR) amplification was conducted with final concentrations of  $1 \mu\text{M}$  primers,  $3 \text{ mM}$   $\text{MgCl}_2$ ,  $0.4 \text{ mM}$  each dNTP, 2.5 units of Taq polymerase (Promega Corp., Madison, WI). Cycling conditions were 40 repetitions of  $95^\circ\text{C}$  for 1 minute;  $50^\circ\text{C}$  for 1 minute;  $72^\circ\text{C}$  for 1.5 minutes with a final extension step at  $72^\circ\text{C}$  for 15 minutes. Amplification was confirmed by agarose gel electrophoresis. PCR amplicons were purified using a QiaQuick PCR purification kit (Qiagen, Valencia, CA), ligated into the pCR<sup>®</sup> II vector and TOP10 cells were transformed according to the manufacturer's instructions (Invitrogen Corp., Carlsbad CA). Transformations were plated onto 2x YT plates containing  $50 \mu\text{g/ml}$  kanamycin and ampicillin (see Appendix 1). White colonies were screened for insert size by PCR amplification. Positive clones

were grown in 2x YT broth with kanamycin and ampicillin. One milliliter of the culture was frozen at -80° C with a final concentration of 25% glycerol and from the remaining 9 ml, plasmid DNA was extracted using a Wizard Plus SV miniprep spin kit (Promega Corp., Madison, WI). These plasmid extracts were sent to the University of Florida's DNA Core Sequencing laboratory to be sequenced.

### Sequence Analysis

Sequences were received from the UF Core laboratory via email and a BLAST search was conducted using GenBank to reveal the orientation of the sequences. The BLAST search also indicated related sequences that would be used in phylogenetic analysis, including the closely related *K. mikimotoi*. Once the orientation was determined, the sequences were imported into the KODON software package version 1.0 (Applied Maths, Inc. Austin, Texas). The sequences were aligned in KODON, which uses a Clustal W-based alignment algorithm (62). The complete multiple sequence alignment from selected algal species covering the 554 base pair (184 amino acid) portion of the *rbcL* gene appears in Appendix 2. Multiple sequence alignments were exported and analyzed using the MEGA 2.0 program (35) for both the amino acid and nucleotide sequences.

The sequence data from the *K. brevis rbcL* clones showed a short (91 bp) region that was markedly different from *K. mikimotoi rbcL* sequence. This portion of the *rbcL* gene of *K. brevis* was selected as the target for a primer and probe set for TaqMan® Taq nuclease assay (Real Time RT-PCR). A primer set and an internal fluorogenic probe

were designed to amplify the 91 bp region (Forward primer: TGAAACGTTATTGGGTCTGT; Reverse primer: AGGTACACACTTTCGTAAACTA; Internal probe: FAM – TTAACCTTAGTCTCGGGTA – TAMRA).

## Testing of the Real Time RT-PCR Primer and Probe Set

### Optimization of Reaction Conditions

The concentration of the primers was the first parameter to be optimized. RT-PCR reactions were set up with the following primer concentrations: 2000 nM, 1000 nM, 500 nM, and 250 nM. In subsequent experiments, the times of the cycle steps (denaturing, annealing and extension) as well as the temperature of the annealing step were varied to determine the optimal cycle conditions. The concentrations of the other reagents in the reaction solution were not optimized, as the one step Real Time RT-PCR master mix kit (Applied Biosystems, Valencia, CA) is pre-mixed for ease of use and increased reproducibility.

### Sensitivity and specificity testing

One of the sequenced clones carrying the 554-bp insert from *K. brevis* APC6 (clone 15) was selected for use in sensitivity testing. Non-target *rbcL* clones from a cruise to the Mississippi River plume in the Gulf of Mexico, known to be amplifiable with other *rbcL* primers surrounding the same region of the gene were used to initially test specificity (See Table 3) (Wawrik, personal communication). Based on the direction of the insert, the vector was linearized by digesting the plasmid with either *HinDIII* or

EcoRV and sense transcript was made by in vitro transcription using the T7 or SP6 promoter site. The transcripts were purified using the Qiagen RNeasy RNA extraction kit with the DNase digestion step according to the manufacturer's instructions (Qiagen, Valencia, CA). These transcripts were quantified using the Ribogreen RNA quantification kit according to the manufacturer's instructions (Molecular Probes Inc., Eugene, OR), mixed 1:1 with RNA storage buffer (8 M guanidinium isothiocyanate, 80 mM Tris-HCl pH 8.5, 24 mM MgCl<sub>2</sub>, 140 mM KCl), aliquoted and frozen at -80°C. The APC6-15 transcript was used to generate Real Time RT-PCR standard curves, while the others were used to test the specificity of the primer/probe set.

RNA was extracted using the Qiagen RNeasy spin kit with the following modifications. One ml of culture was filtered onto a 0.45 µm-pore-size HV polyvinylidene difluoride filter (Millipore Durapore®). The filters were placed into 2 ml screw-cap microcentrifuge tubes containing 750 µl of RLT lysis buffer with 2-mercaptoethanol (10 µl ml<sup>-1</sup>). The filters were incubated for 10 minutes at room temperature and 500 µl were removed into a 1.5 ml microcentrifuge tube and RNA extraction continued according to the manufacturer's instructions (Qiagen, Valencia, CA). The extracted RNA was quantified using the Ribogreen RNA quantification kit according to the manufacturer's instructions. All non-target algal strains were tested for amplification with the real-time primer/probe set with 10 pg of non-target RNA per reaction.

Cell counts for all non-target algal strains as well as *K. brevis* strains were carried out by filtering an appropriate amount of diluted culture onto 0.22 µm black

polycarbonate Poretics filters (Osmonics Inc., Minnetonka, MN). Cells were counted using epifluorescence microscopy on an Olympus BX-60 microscope using appropriate objectives and blue excitation (filter set U-MNIB).

## Results

### Phylogenetic Analysis

A 554 bp *rbcL* amplification product was used for the phylogenetic analysis, which indicated tight clustering of *Karenia* within the Haptophytes at both the protein level and at the nucleotide level (see Figure 3). All *Karenia* were deeply rooted within the form ID *rbcL* group. The *K. brevis* strains were quite different from *K. mikimotoi* ranging from 85% to 86% amino acid similarity and only 76% nucleotide similarity. *K. brevis* strains were very different from other Haptophytes (82% to 85% amino acid similarity), Rhodophytes (79% to 80% amino acid similarity), diatoms (72% to 74% amino acid similarity), *Prochlorococcus* (47% to 49% amino acid similarity) and higher plants (51% to 54% amino acid similarity to Spinach). Table 1 shows the percent identity of *rbcL* sequences from several selected organisms based on at both the amino acid (top) and DNA (bottom) level. Table 2 lists accession numbers for all sequences generated during the course of this study as well as those for the clones used and all other taxa used in the phylogenetic analysis.



Table 1: Percent identity of selected *rbcL* sequences at both the amino acid (top) and nucleotide level (bottom).

	Amino Acid																		
Nucleotide	Appalachicola C6	Piney Island B4	Mexico Beach C5	Charlotte Harbor A2	Charlotte Harbor C2	Karenia brevis	Mexico Beach B3	Karenia mikimotoi	Pleurochrysis	Isochrysis	Prymnesium	Guillardia	Porphyridium	Mazzaella	Gelidium	Rhizosolenia	Heterosigma	Spinacia	Prochlorococcus
Appalachicola C6	98%	98%	97%	98%	97%	96%	85%	83%	82%	83%	76%	80%	79%	79%	72%	76%	51%	47%	
Piney Island B4	99%	100%	99%	100%	99%	98%	86%	85%	84%	84%	78%	82%	80%	80%	73%	77%	53%	48%	
Mexico Beach C5	99%	99%	99%	100%	99%	98%	86%	85%	84%	84%	78%	82%	80%	80%	73%	77%	53%	48%	
Charlotte Harbor A2	98%	99%	99%	99%	99%	98%	97%	86%	84%	83%	84%	77%	81%	80%	80%	73%	77%	52%	48%
Charlotte Harbor C2	98%	99%	99%	99%	99%	98%	86%	85%	84%	84%	78%	82%	80%	80%	73%	77%	53%	48%	
Karenia brevis	98%	99%	99%	99%	99%	97%	86%	85%	84%	85%	78%	82%	81%	81%	74%	78%	54%	49%	
Mexico Beach B3	98%	99%	99%	98%	98%	98%	85%	83%	82%	83%	76%	80%	79%	79%	72%	76%	52%	48%	
Karenia mikimotoi	76%	76%	76%	76%	76%	75%	76%	83%	82%	84%	76%	78%	77%	77%	72%	75%	56%	49%	
Pleurochrysis	73%	73%	73%	73%	73%	73%	73%	73%	92%	92%	85%	86%	84%	84%	78%	83%	52%	50%	
Isochrysis	72%	73%	72%	72%	72%	72%	73%	87%	88%	82%	83%	82%	83%	83%	77%	80%	53%	50%	
Prymnesium	72%	73%	72%	72%	72%	72%	73%	84%	85%	88%	83%	84%	83%	83%	76%	79%	52%	50%	
Guillardia	68%	69%	69%	69%	69%	68%	71%	76%	77%	79%	86%	83%	84%	84%	74%	80%	50%	50%	
Porphyridium	73%	73%	74%	73%	74%	73%	73%	76%	79%	76%	82%	85%	85%	85%	78%	85%	52%	51%	
Mazzaella	74%	75%	74%	74%	74%	75%	74%	72%	74%	75%	77%	78%	80%	88%	78%	82%	51%	50%	
Gelidium	69%	70%	70%	70%	70%	70%	69%	71%	74%	73%	75%	77%	77%	81%	77%	82%	52%	50%	
Rhizosolenia	69%	70%	70%	70%	70%	70%	69%	68%	73%	74%	74%	75%	77%	75%	73%	88%	52%	49%	
Heterosigma	70%	71%	70%	70%	70%	71%	70%	70%	78%	77%	77%	79%	79%	77%	77%	85%	50%	50%	
Spinacia	57%	58%	58%	58%	58%	58%	58%	56%	58%	59%	59%	58%	60%	58%	59%	60%	59%	69%	
Prochlorococcus	54%	55%	55%	54%	54%	55%	55%	55%	54%	55%	58%	58%	59%	61%	59%	58%	58%	63%	

Figure 3: Phylogenetic tree based on *rbcL* amino acid sequences. The tree was obtained from Neighbor Joining analysis using a Poisson distribution of a 148 residue deduced amino acid sequence covering approximately 1/3 of the RuBisCO large subunit. The tree shows RuBisCO form ID diversity and the tight grouping of *K. brevis* and *K. mikimotoi* (both the sequence generated in this study and one from GenBank) within the Haptophyte clade. Several sequences from form IA and IB carrying organisms were used as outgroups. Bootstrap values for 1000 replicates are shown when the value is 40 or higher.

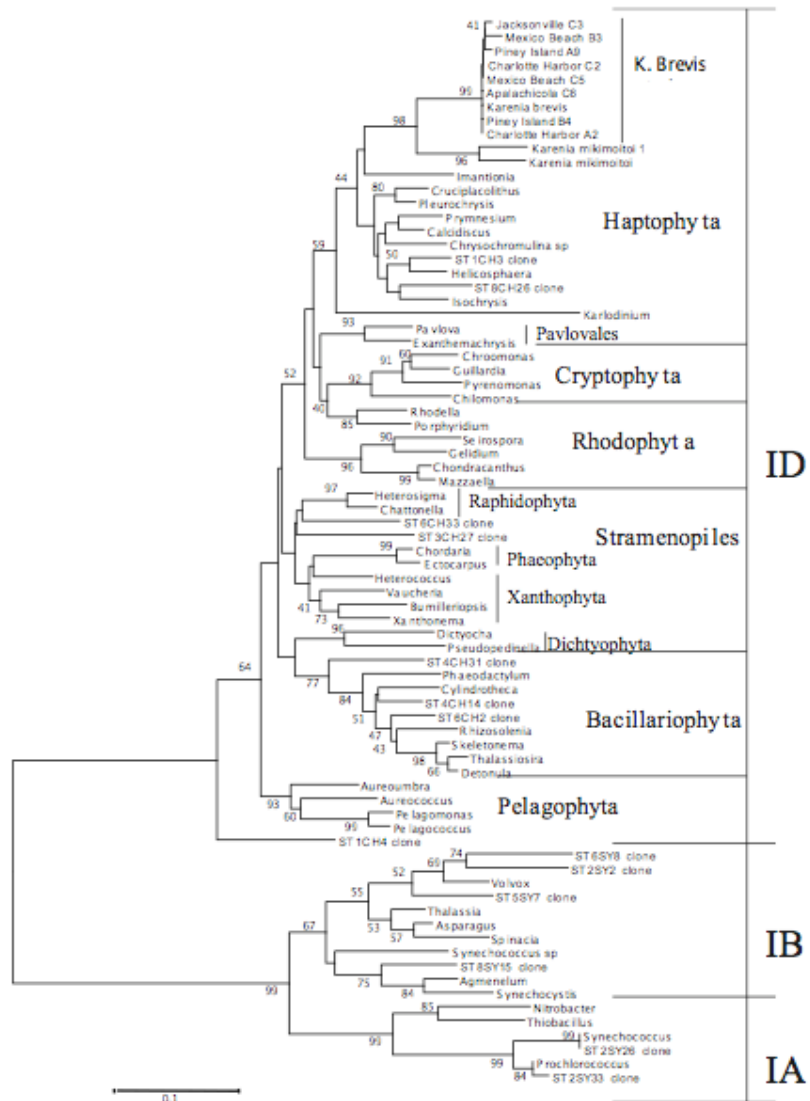


Table 2: Accession numbers for sequences used in phylogenetic analyses.

Species	Strain	GenBank Accession Number
Sequences generated in this study		
<i>K. brevis</i>	APC6	AY154877
<i>K. brevis</i>	CHA2	AY154878
<i>K. brevis</i>	MBC3	AY154879
<i>K. brevis</i>	PIB4	AY154880
<i>K. brevis</i>	CHC2	AY154881
<i>K. brevis</i>	PIA9	AY154882
<i>K. brevis</i>	MBB3	AY154883
<i>K. mikimotoi</i>	N/A	AY251791
Other Sequences used		
<i>Thalassia</i>		AB004897
<i>Detonula</i>		AB018006
<i>Thalassiosira</i>		AB018007
<i>Asparagus</i>		AB029849
<i>Karenia mikimotoi</i>		AB034635
<i>Pleurochrysis</i>		AB043688
<i>Helicosphaera</i>		AB043692
<i>Isochrysis</i>		AB043693
<i>Imantionia</i>		AB043696
<i>Chrysochromulina</i> sp		AB043697
<i>Prymnesium</i>		AB043698
<i>Exanthemachrysis</i>		AB043701
<i>Volvox</i>		AB076099
<i>Rhizosolenia</i>		AF015568
<i>Skeletonema</i>		AF015569
<i>Pelagococcus</i>		AF015580
<i>Chattonella</i>		AF015581
<i>Heterococcus</i>		AF084610
<i>Xanthonema</i>		AF084612
<i>Aureococcus</i>		AF117906
<i>Aureoumbra</i>		AF118136
<i>Mazzaella</i>		AF146214
<i>Phaeodactylum</i>		AF195952
<i>Vaucheria</i>		AF207527
<i>Agmenellum</i>		D13971
<i>Dictyocha</i>		AY043280
<i>Rhodella</i>		AY119776
<i>Chilomonas</i>		AY119780
<i>Chroomonas</i>		AY119781
<i>Pyrenomonas</i>		AY119782
<i>Pavlova</i>		AY119785
<i>Karenia brevis</i>		AY119786
<i>Karlodinium</i>		AY119787
<i>Cylindrotheca</i>		M59080
<i>Prochlorococcus</i>		D21833
<i>Nitrobacter</i>		L22885
<i>Synechocystis</i>		NC 000911
<i>Spinacia</i>		NC 002202
<i>Synechococcus</i> sp		D13539
<i>Synechococcus</i>		U46156
<i>Thiobacillus</i>		X70355
<i>Cruciplacolithus</i>		AB043689
<i>Calcidiscus</i>		AB043690
<i>Ectocarpus</i>		X52503
<i>Heterosigma</i>		X61918

Continued on next page.

---

Table 2: Accession numbers for sequences used in phylogenetic analyses, continued.

---

Species	Strain	GenBank Accession Number
<i>Trichodesmium</i>		AF136182
<i>Amphidinium</i>		AF312209
	ST6SY8 clone	AY157473
	ST8CH26 clone	AY157431
	ST6CH2 clone	AY157408
	ST4CH31 clone	AY157392
	ST4CH14 clone	AY157388
	ST3CH27 clone	AY157382
	ST1CH3 clone	AY157369
	ST5SY7 clone	AY157469
	ST2SY26 clone	AY157446
	ST2SY33 clone	AY157449
	ST2SY2 clone	AY157444
	ST8SY15 clone	AY157479
	ST6CH33 clone	AY157412
	ST1CH4 clone	AY157371

## Real-Time RT-PCR

The TaqMan® probe-based RT-PCR assay (91 bp amplicon) only yielded positive results with *K. brevis* strains (Table 3). All other dinoflagellates (including *K. mikimotoi*) and algal strains resulted in no amplification. All strains tested were present in sufficient concentration to allow for amplification based on the lowest detectable concentration of *K. brevis*.

Standard curves using in vitro transcript from APC6 clone 15 showed linear sensitivity over a range of concentrations spanning 7 orders of magnitude, ranging from  $10^{-1}$  to  $10^5$  fg, as shown in Figure 4. Standard curves using whole-cell extract from *K. brevis* culture were sensitive to as little as 1 pg of total RNA (less than 1 cell reaction<sup>-1</sup>, based on cell counts and dilution) and also showed a linear relationship from 1 cell to 2000 cells reaction<sup>-1</sup> (highest concentration tested). Figure 5 shows an example of the cell standard curves, demonstrating linearity. In addition to showing linearity with both the transcript and whole cellular RNA within each reaction experiment, the assay was relatively consistent between assays. Figure 6 shows a composite average of the transcript standard curves, demonstrating good agreement between amplification events. The composite average of the cell standards curves appears in Figure 7, again demonstrating good agreement between experiments.

Table 3: Positive and negative controls for amplification by real-time RT-PCR

Species	Strain/Clone	Detection by Real-Time PCR
Positive Controls		
<i>K. brevis</i>	Apalachicola B5	+
<i>K. brevis</i>	Apalachicola C6	+
<i>K. brevis</i>	Charlotte Harbor A2	+
<i>K. brevis</i>	Charlotte arbor C2	+
<i>K. brevis</i>	Mexico Beach B3	+
<i>K. brevis</i>	Mexico Beach C5	+
<i>K. brevis</i>	Jacksonville C3	+
<i>K. brevis</i>	Piney Island A3	+
<i>K. brevis</i>	Piney Island B4	+
<i>K. brevis</i>	Wilson	+
Negative Controls		
Dinoflagellates		
<i>K. mikimotoi</i>	CCMP430	-
<i>Amphidinium carterae</i>	CCMP1314	-
<i>Akashiwo sanguinea</i>	CCMP1321	-
<i>Alexandrium tamarense</i>	CCMP1493	-
<i>Glenodinium foliacrum</i>	N/A	-
<i>Gymnodinium catenatum</i>	CCMP1937	-
<i>Gyrodinium</i> sp.	N/A	-
<i>Kryptoperidinium foliaceum</i>	N/A	-
<i>Lingulodinium polyedra</i>	CCMP1738	-
<i>Prorocentrum micans</i>	N/A	-
<i>Scrippsiella trochoidea</i>	N/A	-
<i>Scrippsiella precaria</i>	N/A	-
Diatoms		
<i>Phaeodactylum tricornerutum</i>	CCMP1327	-
<i>Cylindrotheca</i> sp.	ST6CH2 clone	-
<i>Skeletonema</i> sp.	ST4CH31 clone	-
<i>Skeletonema</i> sp.	ST4CH14 clone	-
Raphidophyte		
<i>Heterosigma akashiwo</i>	N/A	-
Praisinophytes		
<i>Tetraselmis</i> sp.	850001	-
<i>Tetraselmis</i> sp.	CCMP961	-
Unidentified sp.	CCMP1536	-
Prymnesiophytes		
<i>Isochrysis</i> sp.	3C	-
<i>Pavlova lutheri</i>	CCMP1325	-
<i>Prymnesium parvum</i>	N/A	-
Unidentified sp.	ST8CH26 clone	-
Unidentified sp.	ST1CH3 clone	-
Chlorophyte		
<i>Chlamydomonas euryale</i>	CCMP219	-
Unidentified sp.	ST5SY7 clone	-
<i>Chlamydomonas</i> sp.	ST2SY2 clone	-
<i>Pycnococcus</i> sp.	ST6SY8 clone	-
Cyanophytes		
<i>Synechococcus</i> sp.	CCMP836	-
<i>Synechococcus</i> sp.	WH7803	-
<i>Synechococcus</i> sp.	ST2SY26 clone	-
<i>Prochlorococcus</i> sp.	ST2SY33 clone	-

Continued on next page.



Table 3: Positive and negative controls for amplification by real-time RT-PCR, continued

Species	Strain/Clone	Detection by Real-Time PCR
<i>Trichodesmium</i> sp.	ST8SY15 clone	-
Trebouxiophyte		
<i>Chlorella autotrophica</i>	CCMP243	-
Coccinodiscophyte		
<i>Thalassiosira pseudonana</i>	CCMP 1335	-
Eustigmatophyte		
<i>Nannochloropsis</i> sp.	ST3CH27 clone	-
<i>Nannochloropsis</i> sp.	ST1CH4 clone	-
Xanthophyte		
<i>Heterococcus</i> sp.	ST6CH33 clone	-

Figure 4: An example standard curve generated using in vitro transcript. The curve is plotted with the trendline indicating the precision of the real-time RT-PCR assay.

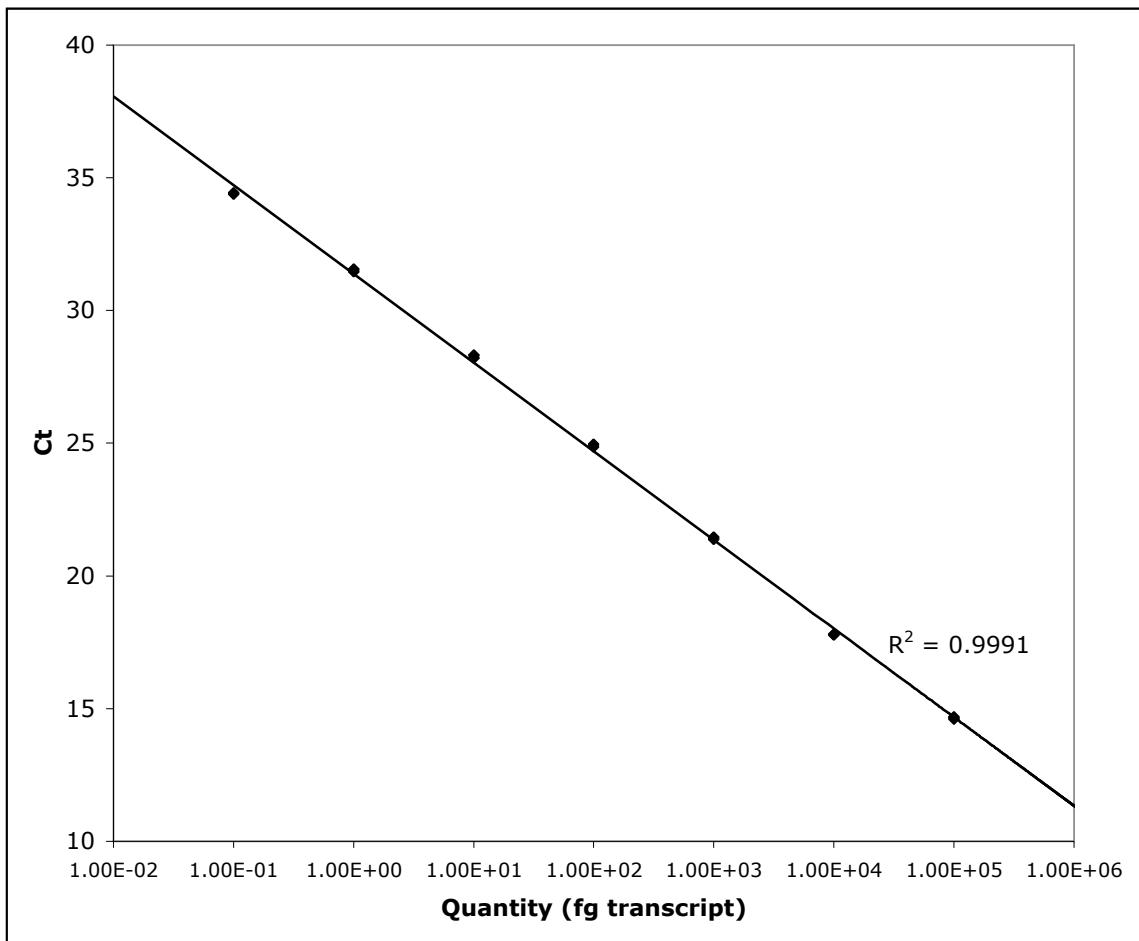


Figure 5: An example standard curve generated using whole cell RNA extract. The curve is plotted with a trendline indicating the precision of the real-time RT-PCR assay.

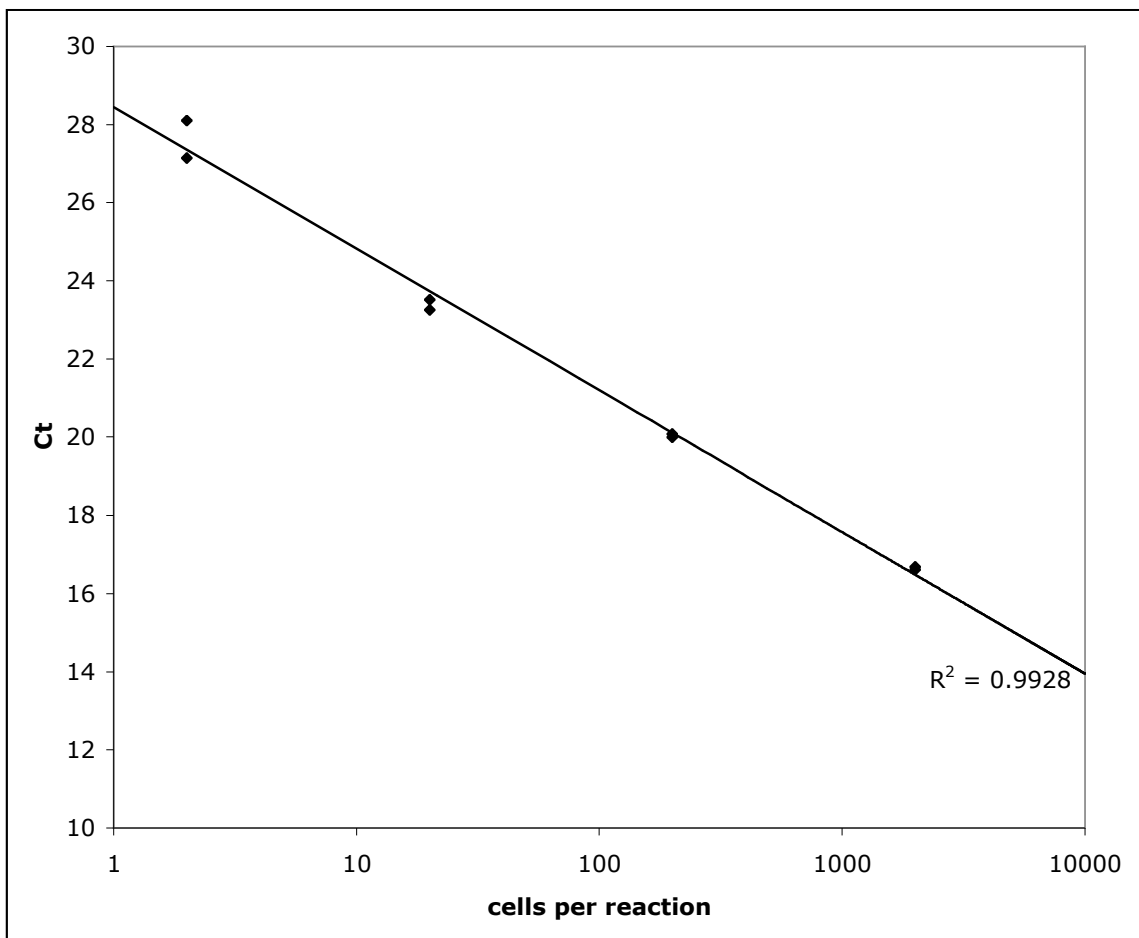


Figure 6: Composite of all transcript standard curves. Error bars represent the standard deviation of the Ct values for each of the transcript concentrations (n=30).

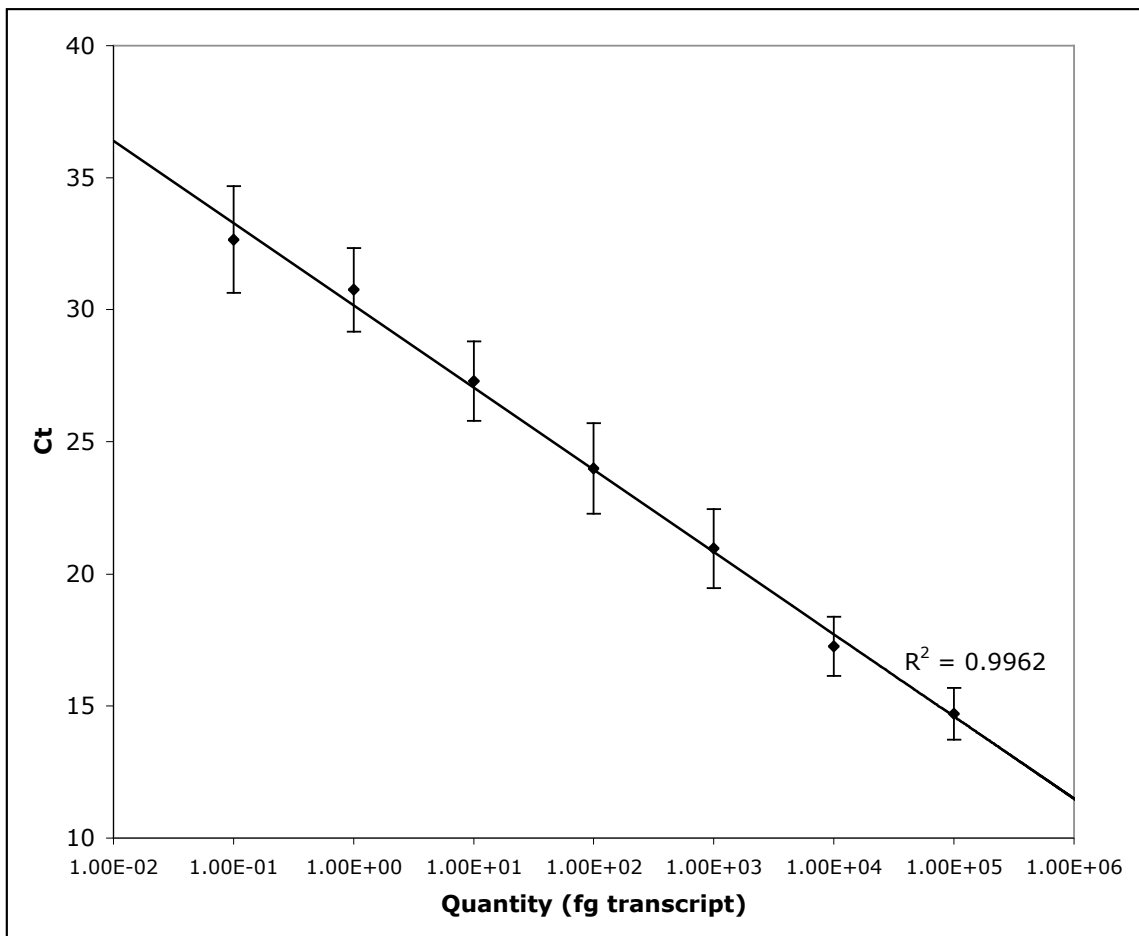
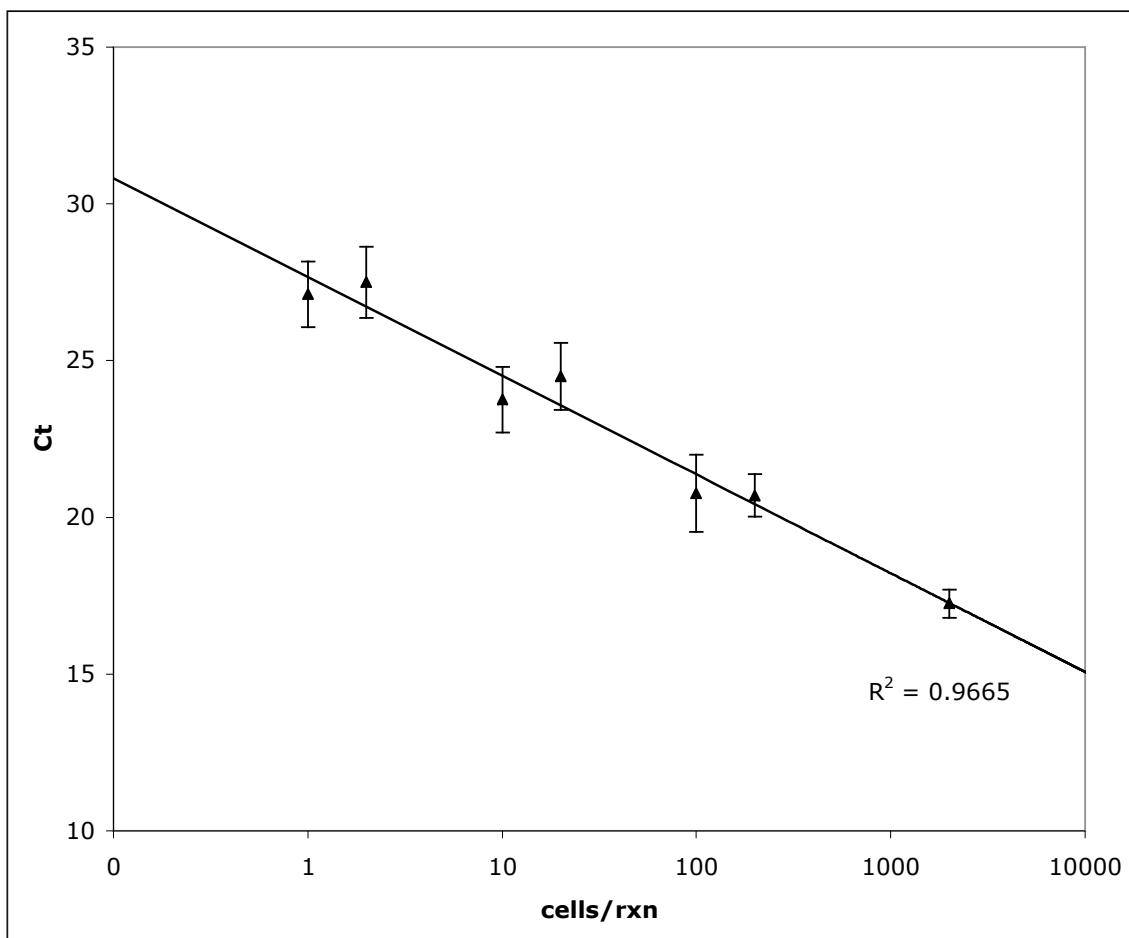


Figure 7: Composite of all cell standard curves. Error bars represent the standard deviation of the Ct values for each of the cell concentrations (n=10).





## Discussion

A real-time RT-PCR method was developed to detect and quantify cell abundance based upon *rbcL* transcription in the red tide forming dinoflagellate *K. brevis* with good specificity and sensitivity. Using our TaqMan® probe, we were able to amplify and detect a wide range of concentrations of *K. brevis* to the exclusion of all non-target DNA and RNA tested. The dynamic range over which this technique is effective covers the range of natural *K. brevis* blooms in the environment. Populations of *K. brevis* with <1000 cells L<sup>-1</sup> (as determined by microscopy cell counts) is considered “present” but poses no risk of adverse health effects or shellfish contamination. Samples with <5000 cells L<sup>-1</sup> are considered to have a “Very Low” level bloom, carrying a slight risk of respiratory irritation. When concentrations of >5000 cells L<sup>-1</sup> occur, shellfish harvesting is closed. The highest level of a bloom has been reached when there are >1 million cells L<sup>-1</sup>. A bloom of this magnitude can result in massive fish kills, respiratory distress, discoloration of the water and may have effects on higher animals such as dolphins and manatees. These ranges are well within the range of detection ability of this assay when as little as 1-5 ml of sample water is filtered and extracted.

It is essential that a probe designed to detect an important bloom forming organism not detect other closely related (or unrelated) organisms. Bloom detection and tracking relies heavily on being sure of the identity of the blooming organism. The TaqMan® probe created in this study showed high specificity by only amplifying *K. brevis* and not amplifying any of the non-target samples tested, as listed in Table 3.

This method represents the first molecular detection strategy for *K. brevis*, and it may be well suited for detection and monitoring of red tide blooms caused by *K. brevis* in the Gulf of Mexico and the southern Atlantic coast of the United States. It may provide an easy and relatively rapid procedure that could be employed as an alternative to the more difficult and time-consuming methods currently used by red tide monitoring and management programs in the state of Florida and other states affected by *K. brevis*.

## **Chapter 3: Testing of the assay, field samples, diel studies and method evaluation**

### **Introduction**

The real-time RT-PCR detection and enumeration strategy outlined in the previous section performed well under preliminary testing conditions. However, an assay is only as good as its applicability to natural environmental samples. For a *K. brevis* detection assay to have full applicability, it has to accurately and reliably detect and enumerate populations of *K. brevis* from natural samples and it must be robust enough for much of the assay to be conducted in non-laboratory conditions. Specifically, the RNA purification has to be complete enough to allow for reliable detection of *K. brevis* regardless of the other constituents of the water sample to be analyzed, such as other phytoplankton species and organic detritus. The extraction procedure must also allow for the possible lack of adequate facilities that may be encountered either shipboard or in other field situations. At this time, unfortunately, the real-time RT-PCR step is constrained mostly to the lab due to the required instrumentation, such as the Applied Biosystems model 7700 Sequence Detection System used in this study; however there are systems that are beginning to show some field deployable capabilities.

In addition to being able to reliably enumerate *K. brevis* from natural samples despite possible interference, the assay may be sensitive to cell phase. Dinoflagellates

have been shown to exhibit diel cell cycling with certain genes and enzymes. In *K. brevis*, cell cycling over diel periods had been demonstrated and linked to cyclin-dependant kinase (66), with the dark to light transition shown to be the dominant cue. Expression of some genes in some dinoflagellates has been shown to be regulated over a diel cycle. Approximately 3% of the genes in *Pyrocystis lunula* were shown to be circadian regulated at the transcriptional level (44), notably several genes responsible for translation demonstrated diel regulation, but regulation of carbon fixation gene expression has so far not been shown in dinoflagellates. RuBisCO appears not to be differentially expressed at the mRNA level nor do protein levels change in the dinoflagellate *Lingulodinium polyedrum* over the diel cycle (44) and differences in photosynthetic capacity appear to be controlled by differential distribution of RuBisCO within the chloroplasts of *Gonyaulax*, with protein levels remaining constant (42). It has, however been shown that *rbcL* expression can exhibit great diel variability in other organisms, such as diatoms (*Phaeodactylum tricornutum*) (67), where transcript levels varied by a factor of approximately 3. It would be important to know for this assay if correction needs to be made based on time of day that the sampling was carried out, in order for the counts inferred from the real-time RT-PCR to more accurately represent the actual cell concentrations, should there be any diel variation in transcription of *rbcL*.

## Methods

### RNA extraction and amplification from environmental samples

Environmental bloom and non-bloom samples were received from Earnest Truby at the Florida Fish and Wildlife Conservation Commission's Florida Marine Research Institute (FMRI). *Karenia brevis* abundance in samples was determined by microscopy by FMRI personnel (Table 4).

Ten to 20 ml of the samples were filtered onto sterile 0.45  $\mu\text{m}$  pore-size Millipore™ Durapore® type HVLP filters and placed in lysis buffer (buffer RLT, Qiagen, Valencia CA or Lysis buffer, Stratagene, La Jolla, CA) and processed immediately if possible. If immediate processing was not possible, the tubes were frozen at  $-80^{\circ}\text{C}$ . Samples were processed using the RNA extraction kits from either Qiagen (RNeasy™) or Stratagene (Absolutely RNA™) according to the manufacturer's instructions. From the extraction (50  $\mu\text{l}$  final eluent), 5  $\mu\text{l}$  were added to the amplification reaction and real-time RT-PCR was carried out as described in the previous section with a standard curve of transcript spanning seven orders of magnitude (0.1 to  $10^5$  fg of target transcript reaction<sup>-1</sup>) and a standard curve of whole cell extracts spanning 3-4 orders of magnitude (i.e. 1 to 1000 cells per reaction). Real-time RT-PCR data generated from these amplifications was analyzed preliminarily using the Sequence Detection software (versions 1.6 to 1.9) supplied by Applied Biosystems. Further analysis was carried out using Excel (Microsoft Corporation, Redmond, WA).

## Diel experiments

### Cultures and culture conditions

For all diel experiments the same isolate, Piney Island B4 was used. The cultures were acquired from Bill Richardson at FMRI and placed in an incubator in our lab at 22° C under a 12-hour light-12-hour-dark regimen at 26  $\mu\text{mol s}^{-1} \text{m}^{-2}$  in F/2-Si medium (18) for at least 3 days to acclimate before any experimentation.

### Sampling schedule, cell counts and RNA extraction

Four non-consecutive 24-hour diel studies were conducted as follows (using a new culture each time): starting at 4:00 AM, before the lights of the incubator were turned on (at 6:30 AM) and every two hours thereafter, the culture was removed from the incubator, gently swirled to homogenize the culture without lysis of the fragile *K. brevis* cells and approximately 30 ml of culture were poured into a 50 ml disposable centrifuge tube. All further sampling (cell counts, RNA extraction and carbon fixation) was carried out using sub-samples from this 30 ml sample. Before every removal of sub-samples from the 30 ml aliquot, the 50 ml centrifuge tube was capped and gently inverted to ensure even distribution of the cells and wide-bore pipette tips or serological pipettes were used to decrease turbulence and accidental cell rupture.

Cell counts were carried out as described in the last section (briefly, filtering onto a 0.22  $\mu\text{m}$  pore-size black membrane and counted by epifluorescence microscopy, in triplicate).

Duplicate or triplicate 1 ml culture samples were filtered onto sterile Durapore<sup>®</sup> filters and placed in Qiagen's RLT or Stratagene's Lysis Buffer and immediately frozen

in liquid nitrogen and placed in the freezer at  $-80^{\circ}\text{C}$  for extraction and real-time RT-PCR analysis once the diel sampling period was over. These samples were extracted, amplified and analyzed as described above.

### Carbon fixation

Two 10 ml aliquots of culture were placed into acid washed, sterile scintillation vials, one clear glass and the other covered with black electrical tape to seal out light. To each of these aliquots,  $5\ \mu\text{l}$  of  $^{14}\text{C}$  labeled  $\text{HCO}_3^-$  ( $10\ \mu\text{Ci}$ ) were added. Two 2 ml samples from each vial were immediately filtered onto  $0.22\ \mu\text{m}$  pore-size Millipore type GS filters, rinsed with filtered seawater and placed in scintillation vials with 0.5 ml of 0.5 N HCl. The clear vial was placed in a lighted incubator at room temperature with  $30\ \mu\text{mol s}^{-1}\ \text{m}^{-2}$  light and the taped (dark) vial was placed in a dark drawer at room temperature. The vials were incubated for 30 minutes. After the incubation, two 2 ml aliquots were again filtered from each sample, rinsed with filtered seawater and placed in scintillation vials with 0.5 ml of 0.5 N HCl. Once all of the time points had been sampled, the vials were allowed to remain at room temperature for 8 to 10 hours to allow unincorporated  $^{14}\text{C}$  (as  $\text{CO}_2$ ) to off-gas. To each of the samples, 1 ml of ethyl acetate was added and the filter was allowed to dissolve for 1 hour. Then 10 ml of Ecoscint A scintillation fluid (National Diagnostics, Atlanta, GA) was added, and the samples were placed into the scintillation counter to be counted for 0.5 minutes each.

Counting efficiency was determined as follows: a Millipore GS filter was placed in 3 scintillation vials and dissolved with 1 ml ethyl acetate. Ten ml of Ecoscint A and 1



ml of 0.01 N NaOH was added to each vial and mixed well. To two of these vials, 5  $\mu$ l of diluted  $^{14}\text{C}$  was added (diluted 1:1000, 0.01  $\mu\text{Ci}$ , 22,200 DPM) and mixed well, the third vial served as a blank for background comparison. These vials were also counted for 0.5 minutes each. Efficiency (e) was calculated using the following equation:  $e = (\text{CPM}_{\text{sample}} - \text{CPM}_{\text{blank}}) / 22,200$ . Counting efficiency ranged from 0.30 to 0.70. These data were analyzed either by the C14 program (Qbasic, in-house software) or using an Excel spreadsheet based on that program.

#### Alkalinity Determination

After the initial sampling, 100 ml of culture were removed and analyzed for culture alkalinity as follows. From the 100 ml sample, the temperature, salinity and pH were determined. The sample was placed on a stir-plate with a stir-bar and 25 ml of 0.01 N HCl were added and the pH was measured again. If the pH was not below 4.0, an additional 5 ml of 0.01 N HCl were added and the pH was measured. The data from these measurements were analyzed by the ALK1 program (Qbasic, in-house software) for an estimated alkalinity and amount of dissolved inorganic carbon. These data were used in the  $^{14}\text{C}$  incorporation calculations.

#### Evaluation of real-time assay reagents and extraction methods

##### Room temperature and freezer (-80° C) stability of *K. brevis* RNA lysates

In order to determine the field applicability, convenience and flexibility inherent in the real-time RT-PCR assay, a series of experiments were carried out based on anticipated field extraction conditions. To determine the stability of the target RNA in

cases where liquid nitrogen or  $-80^{\circ}\text{C}$  freezers are not readily available, and to evaluate the performance of the guanidine thiocyanate-based lysis buffers from both Qiagen and Stratagene, two separate experiments were carried out. In the first, 20 1-ml aliquots of culture were filtered onto Durapore<sup>®</sup> filters and placed in RLT buffer with 2-mercaptoethanol. Two of those samples were frozen immediately at  $-80^{\circ}\text{C}$  and the rest were placed in a dark drawer at room temperature. Each day, 2 of the samples were removed from the drawer, labeled with the day and frozen. Once all 10 days worth of samples had been frozen, the samples were thawed and extracted following Qiagen's instructions. The samples were then analyzed by real-time RT-PCR as described above. The second experiment was conducted as above using Stratagene's Lysis Buffer and extraction kit, but the sampling was done in quintuplicate.

To evaluate the samples' stability in Stratagene's Lysis buffer at  $-80^{\circ}\text{C}$ , 25 1 ml aliquots were filtered onto Durapore<sup>®</sup> filters and placed into lysis buffer. Twenty of the samples were placed immediately into the  $-80^{\circ}\text{C}$  freezer, while the remaining 5 were extracted and analyzed using the real-time RT-PCR assay. At the following intervals 5 of the samples were thawed, extracted and analyzed using the real-time RT-PCR assay: 1 week, 2 weeks, 4 weeks and 6 weeks.

#### Alternative extraction methods

In order to evaluate and minimize variation due to the RNA extraction process, several approaches were tested. As the *K. brevis* cell is very fragile, two methods were tested to minimize breakage and subsequent loss of RNA and one method was tested to

reduce sample handling and possibly lead to a more easily automated extraction protocol. In the first method, rather than filtering onto Durapore<sup>®</sup> membranes, 1 ml of culture was centrifuged at 5000 rpm (2300 X g), the supernatant was removed and the cells were resuspended in 100  $\mu$ l of lysis buffer. Extraction was continued according to the manufacturer's instructions. In the second method, rather than manipulating the cells at all, 1 ml of culture was added directly to 1.5 ml of lysis buffer and vortexed vigorously. To this mixture, 2.5 ml of 70% ethanol was added and the samples were run through the RNA extraction column either through multiple centrifugation steps or pulled through by placing the column in a centrifuge tube with the bottom cut off and placed into a rubber stopper that was placed on a vacuum manifold and light vacuum was applied (80 to 100 mm Hg). Extraction was continued according to the manufacturer's instructions. The third method tested was designed to facilitate future automation of the extraction process and was carried out by filtering the *K. brevis* culture directly onto the silica matrix of the extraction column using a light vacuum as above. To the column, 200  $\mu$ l of a 1:1 mixture of lysis buffer and 70% ethanol were added, incubated for 10 minutes and removed by centrifugation. Extraction was continued according to the manufacturer's instructions. The standard method using the Durapore<sup>®</sup> membrane was also conducted for comparison. All culture samples were diluted so that 1000 cells were extracted into the final eluent, and extractions were performed with the Stratagene columns with five replicates of each extraction method.

## Results

### Field samples

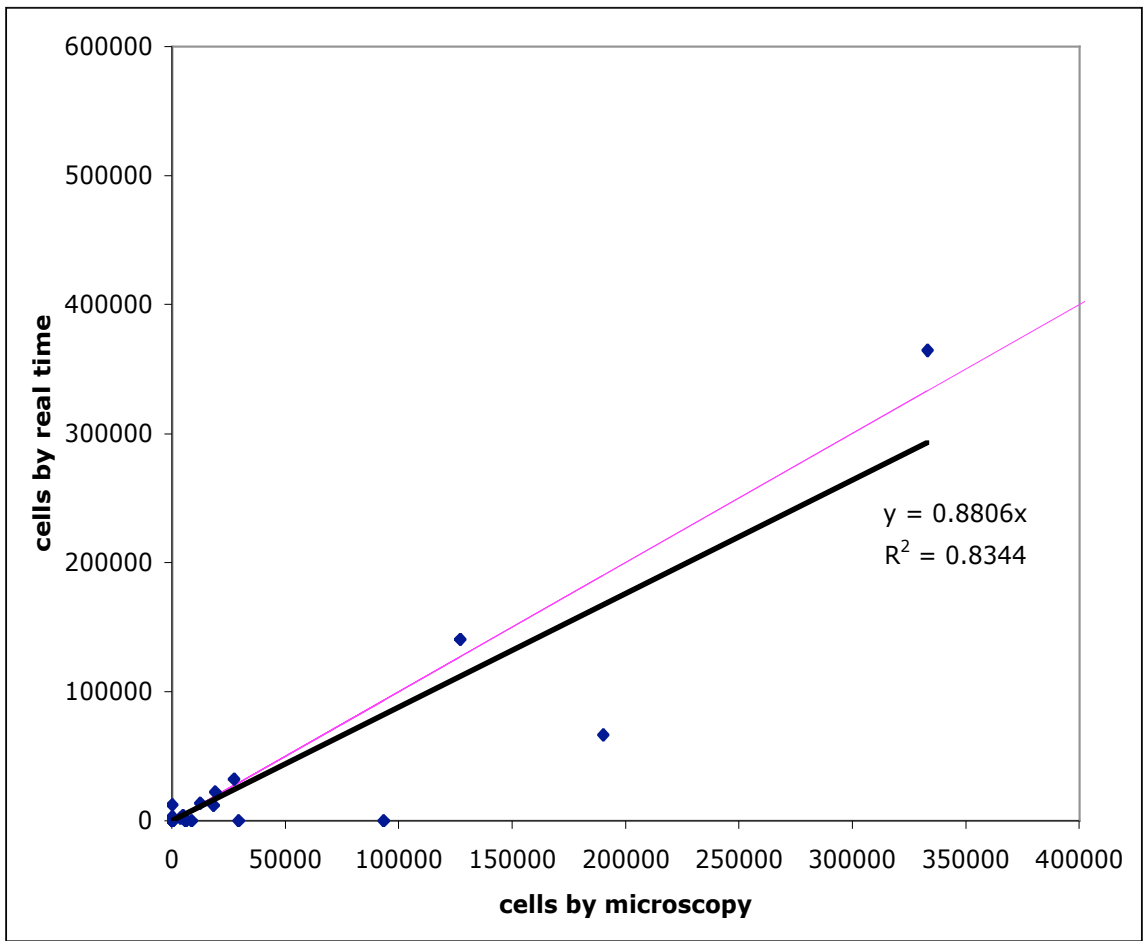
Twenty-eight field samples from FMRI were analyzed by the real time RT-PCR assay and the calculated cell counts based on the cell and transcript standard curves as well as the microscopy counts are listed in Table 4. Figure 8 shows a graphical comparison of the microscopy-based counts and the real-time RT-PCR assay, with a 1:1 line plotted for reference. The correlation ( $r^2$ ) between microscopy counts and real-time RT-PCR counts was 0.8344, with a slope of 0.88. Samples that were negative by microscopy (limit of detection approximately 300 cells liter<sup>-1</sup>) often were positive by RT-PCR (ranging from 0 to 12476 cells liter<sup>-1</sup>, average: 1646 cells liter<sup>-1</sup>, n= 12). The samples that were stored in the freezer for over a month (bloom dates 7/1/03 and 8/27/03) yielded dramatically lower calculated cell concentrations than expected, indicating background concentrations of *K. brevis* despite the microscopy counts that were in the 6,000 to 100,000 cells L<sup>-1</sup> range as counted prior to filtering and freezing in lysis buffer

Table 4: Bloom samples counted by microscopy and inferred counts by real-time RT-PCR.

Site ID	Sampling date	Cell Counts (Cells/L)	Real Time PCR (Cells/L)	Bin agreement
Naples Pier	3/26/03	0	219	Y
Clam Pass	3/26/03	12300	13752	Y
Barefoot Beach	3/26/03	333000	364645	Y
Naples Pier	4/2/03	190000	66540	N
South Marco	4/2/03	0	1791	N
951 Launch ramp	4/2/03	0	1152	N
Clam Pass	4/2/03	127000	140758	Y
Good Land	4/2/03	0	3167	N
Marco Pass	4/2/03	0	12476	N
Howard Park	7/1/03	0	0	Y
Honeymoon Island	7/1/03	6000	12	N
Pier 60	7/1/03	6330	634	N
Reddington Pier	7/1/03	0	2133	N
Bowditch Park	8/27/03	29300	133	N
Bonita Beach Park	8/27/03	0	4	Y
Lover's Key	8/27/03	93300	122	N
Little Hickory Island Park	8/27/03	8670	49	N
Lely Barefoot Beach	10/16/03	4670	4089	Y
Seagate	10/16/03	27300	32311	Y
Naples Pier	10/16/03	0	0	Y
Vanderbilt Beach	10/16/03	19000	22484	Y
South Marco Beach	10/16/03	18300	12050	Y
Naples Pier	10/27/03	3670	2295	Y
Seagate	10/27/03	667	294	Y

Bin Key	<i>K. brevis</i> cells/l
Present	<1000
Very Low a	>1000 to 5000
Very Low b	5000 to 10,000
Low a	10,000 to 50,000
Low b	50,000 to 100,000
Medium	100,000 to 1,000,000
High	>1,000,000

Figure 8: Cell counts microscopy and the real-time RT-PCR assay. Comparison of counts by microscopy and inferred counts by real-time RT-PCR. Plotted are both a trendline for the data (thick line) and a one-to-one line (thin line) that would indicate perfect agreement, for comparison.





## Diel Studies

Carbon fixation showed a typical diel pattern of increased production during the day, with a peak of production occurring around noon. Production increased rapidly after the start of the light period, beginning with the 8:00 AM sampling period for all of the diel studies, and had reached maximal activity by the noon sampling. In 2 of the 4 diel periods studied, *K. brevis* displayed a depression in carbon fixation at the 2:00 PM sample, but had returned to a higher production level at the 4:00 PM or 6:00 PM sampling. In the other two diel studies, the *K. brevis* cultures did not express this decline in production during the light period. Carbon fixation returned to nighttime levels by 8:00 PM, 1.5 hours after the start of the dark period. Figure 9 shows carbon fixation over the four non-consecutive 24-hour periods analyzed.

Cell counts during the 24 hour sampling periods of the four diel studies oscillated, varying by as much as 2000 cells ml<sup>-1</sup>, most likely due to incomplete mixing of the culture before the initial sampling of the 30 ml aliquot. The cultures did not display significant growth over the 24-hour periods studied, and there was no trend to the variation in cell counts.

Expression of the *rbcL* gene as analyzed by real-time RT-PCR showed some variability over the diel period in each of the 24-hour periods studied, however these variations do not fall at the same time periods in all of the 4 diel periods studied. Figure 10 shows the *rbcL* RNA signal as detected by real-time RT-PCR, corrected by cell counts at each time point, where possible. Figure 11 shows an overlay of all of the diel RNA

Figure 9: Carbon fixation over one diel cycle in four experiments. Diel experiments 1 through 4 are represented by diamonds, squares, triangles and x marks, respectively.

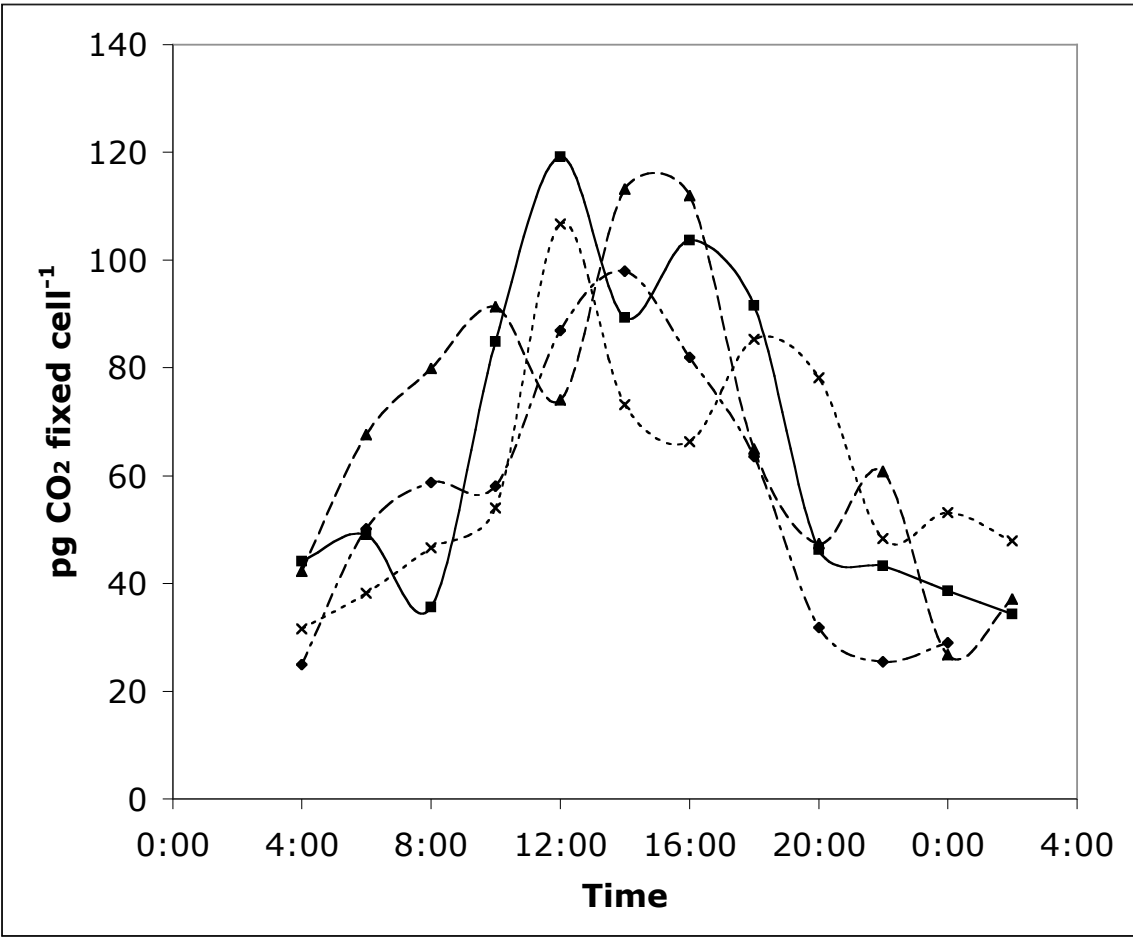


Figure 10: RNA transcription over the diel periods. Diel experiments 1 through 4 are represented by diamonds, squares, triangles and x marks, respectively.

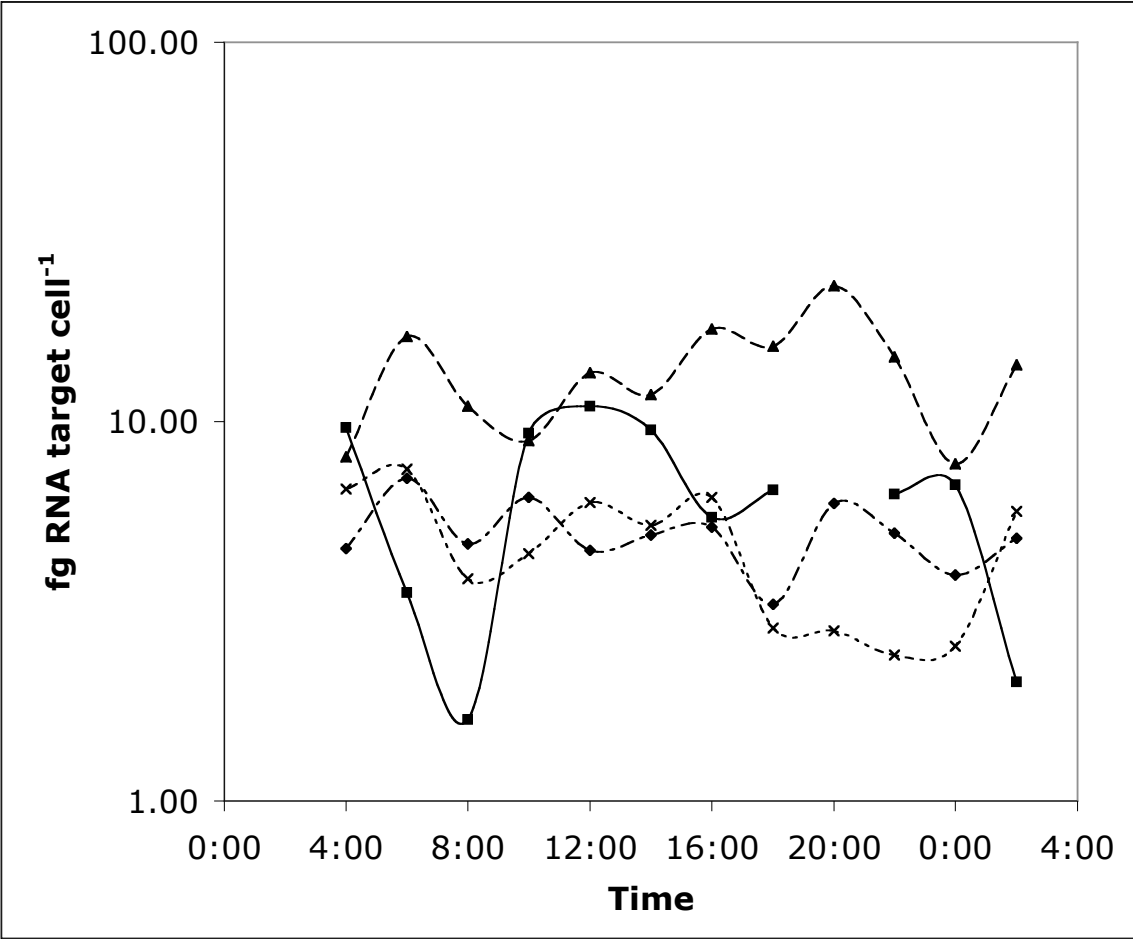
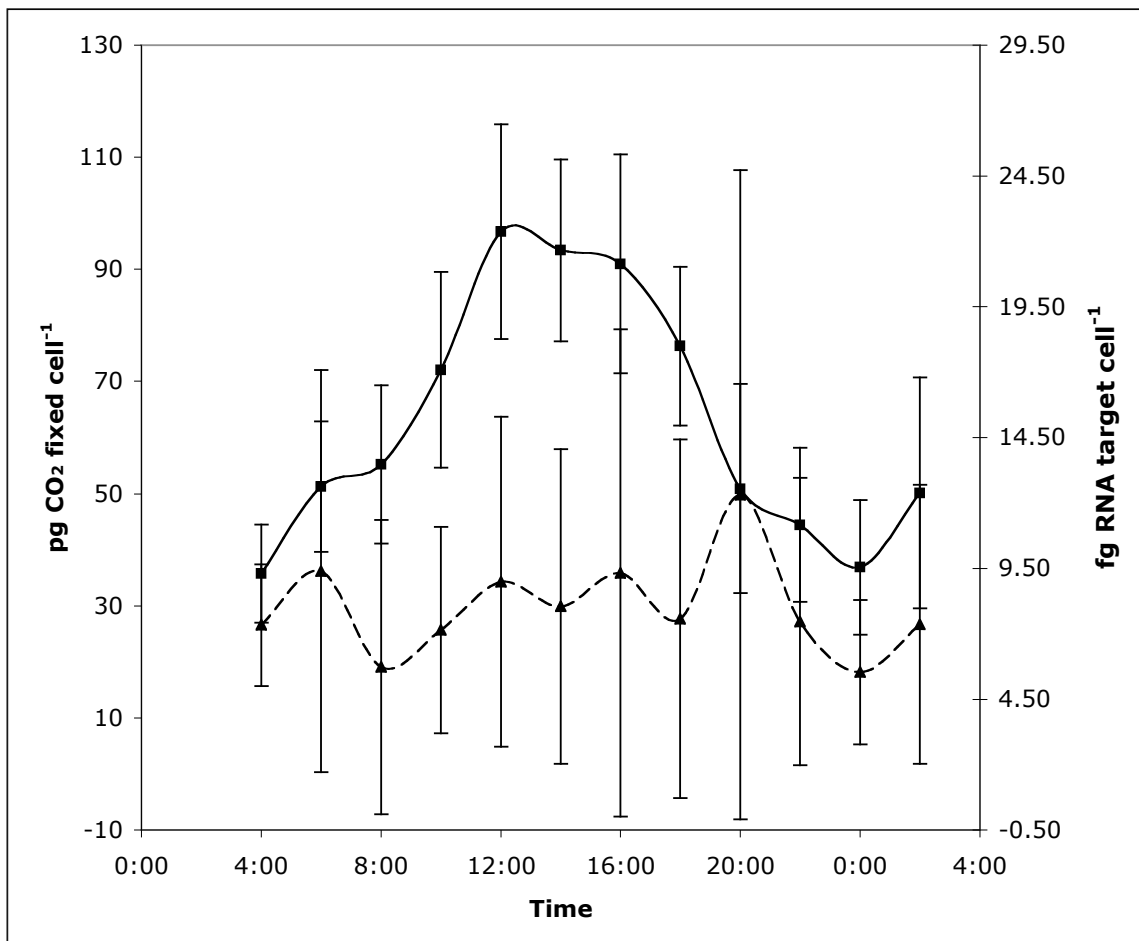


Figure 11: Average carbon fixation (squares, solid line) and average *rbcL* transcription (triangles, dashed line) for all of the diel experiments.



results and the carbon fixation results. The minima and maxima in RNA transcript levels did not consistently occur at any specific time in the diel cycle and ranged from 3.30 to 7.09 fg cell<sup>-1</sup>, 1.64 to 10.96 fg cell<sup>-1</sup>, 7.72 to 22.79 fg cell<sup>-1</sup> and 2.43 to 7.48 fg cell<sup>-1</sup> in each of the diel experiments, with an average range of 5.53 to 12.31 fg cell<sup>-1</sup> and an average cellular *rbcL* target concentration of 7.50 fg cell<sup>-1</sup>.

#### Room Temperature Stability in Lysis Buffer

Samples retained much of their RNA when stored in lysis buffer from Stratagene when stored at room temperature in the dark for as much as 6 days, however the replicates became more variable in terms of their RNA content. On average, the RNA content did not dramatically change, however, by day seven, there were samples in which very little (two orders of magnitude less) RNA remained. Samples stored in Qiagen's lysis buffer lost approximately half of the RNA after one day stored at room temperature, but remained at that lower level for 6 more days before continuing to degrade. Figure 12 shows the RNA present in samples stored at room temperature in the dark in Qiagen's lysis buffer (Figure 12(a)) and Stratagene's lysis buffer (Figure 12(b)) and the stability of the target over 10 days. Figure 13 compares the change in RNA signal in the two lysis buffers, normalized as average percent change of RNA concentration from day 0.



### Freezer stability in lysis buffer

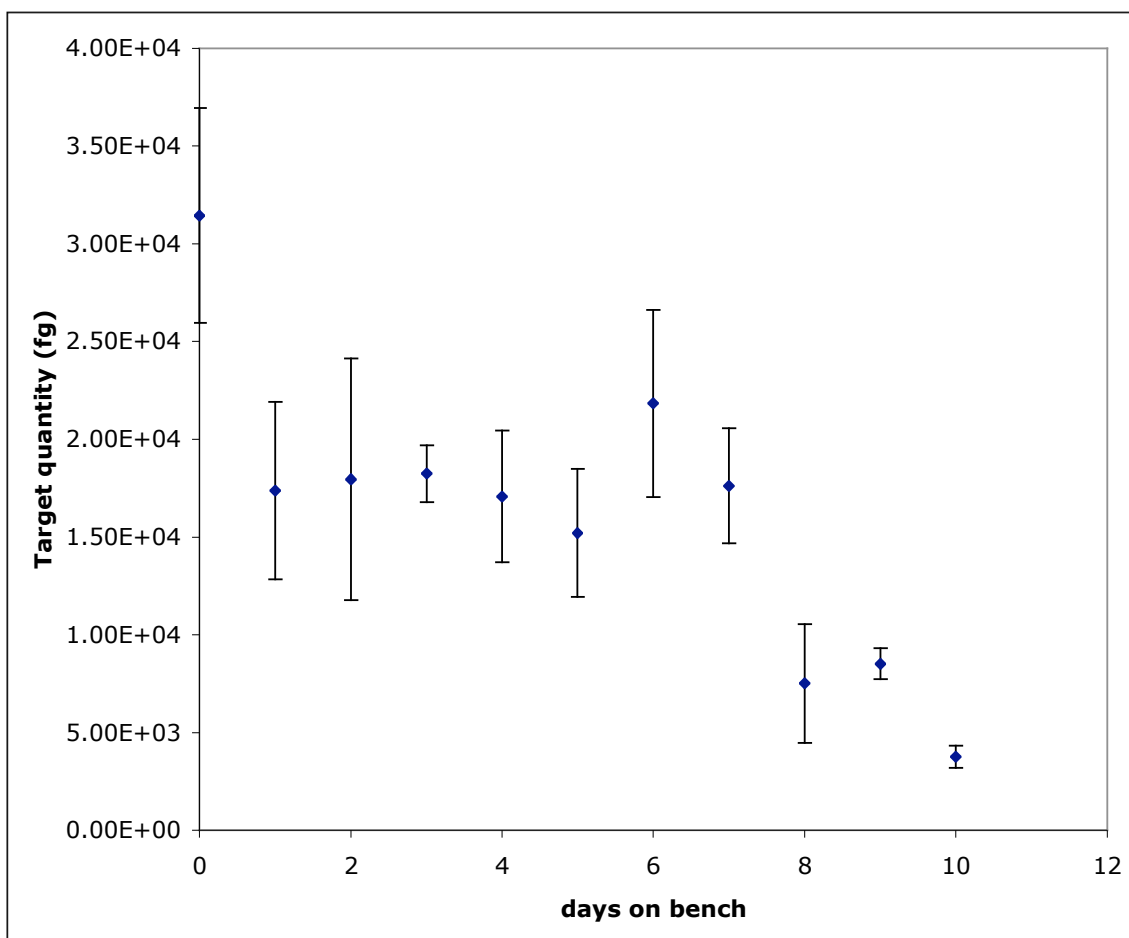
Samples stored in Stratagene's lysis buffer at  $-80^{\circ}\text{C}$  began to lose some target RNA content after two weeks, though with less variability than was seen associated with the room temperature-stored samples after 7 days. The loss of RNA seen in the samples at three weeks did not continue as a trend and at the end of the experiment there was not a significant loss of RNA in the samples stored for as much as six weeks in the  $-80^{\circ}\text{C}$  freezer. Figure 14 shows the detected amount of RNA from samples stored at  $-80^{\circ}\text{C}$  over the course of six weeks.

### Alternative extraction methods

Samples analyzed with the alternative extraction methods, though representing the same cell numbers, did show differences in their RNA signal. Samples that were filtered directly onto the extraction column, without prior filtration or concentration steps returned on average the most RNA of the methods tested, however the column filtration method also exhibited the most variability of RNA signal. The two methods which used a concentration mechanism (filtration onto a Durapore<sup>®</sup> membrane or concentration by centrifugation) displayed very similar RNA signals, with the centrifugation method showing less variability than the filtering method. The extraction directly from the liquid culture returned the lowest RNA signal, as well as the lowest variability. The results of this experiment are presented in Figure 15.

Figure 12: RNA stability at room temperature. (A) RNA stability at room temperature in Qiagen's RLT buffer. Plotted are the mean values and the error bars represent the standard deviation (n=3). (B) RNA stability at room temperature in Stratagene's Lysis buffer. Plotted are the mean values and the error bars represent the standard deviation (n=5).

A)



B)

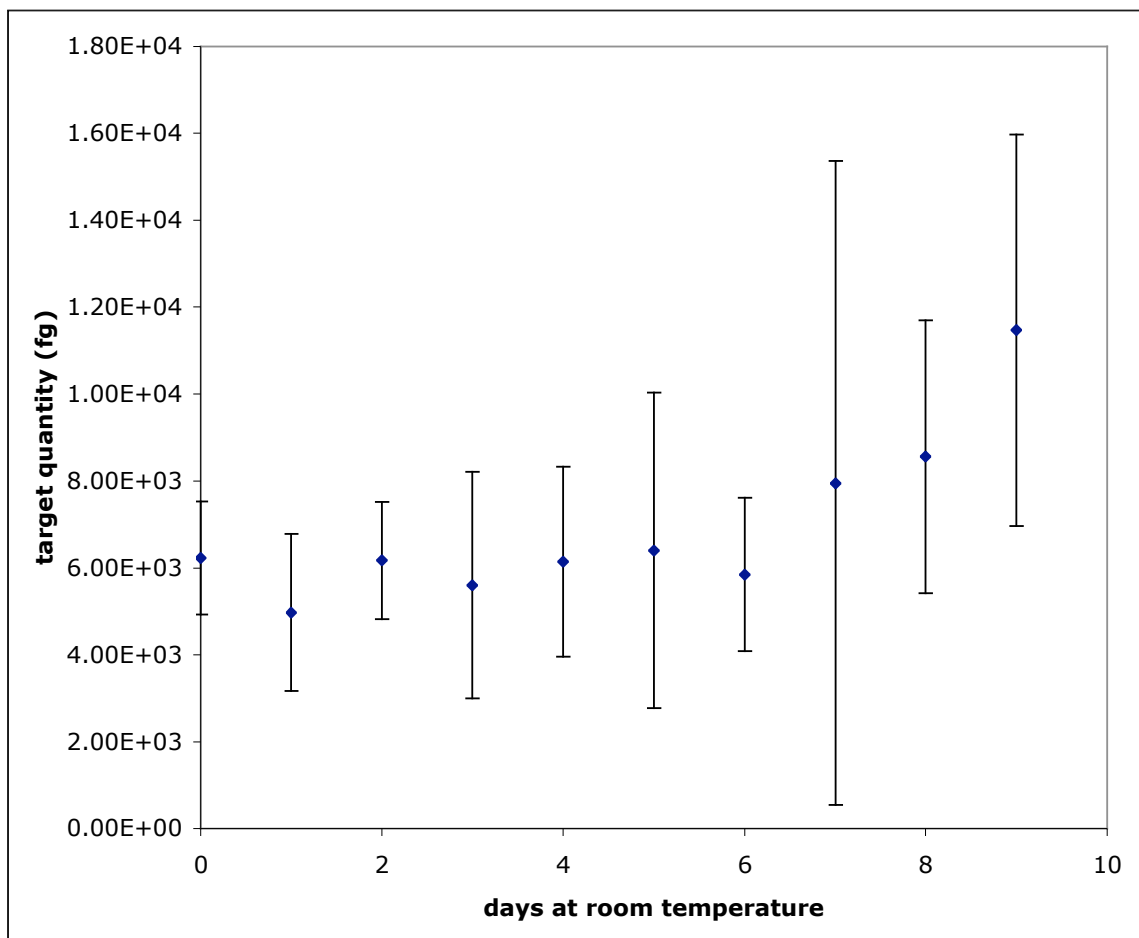


Figure 13: Average percent of initial RNA signal after storage at room temperature. Shown are the average percents of the detected *rbcL* RNA signal after storage at room temperature in RLT (squares) and Lysis buffer (circles).

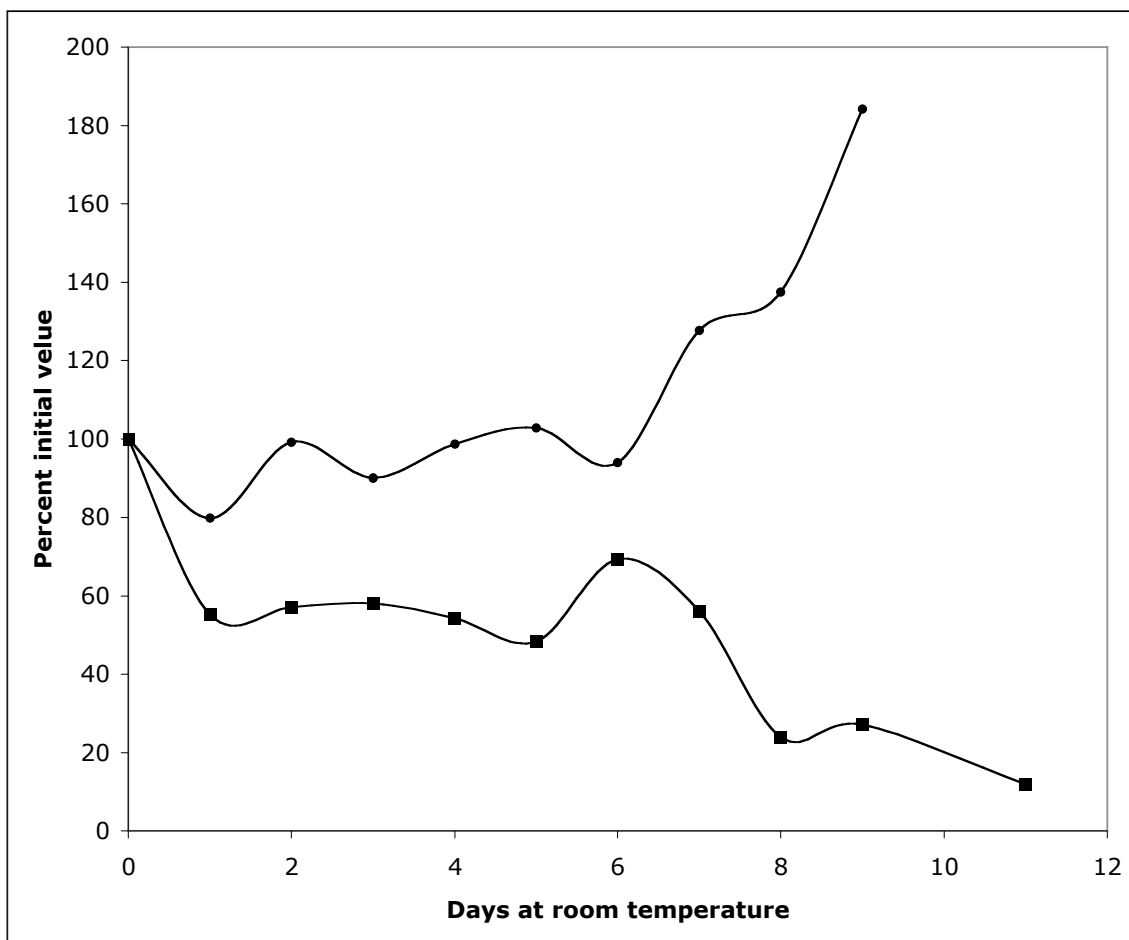


Figure 14: RNA stability in Stratagene's Lysis buffer after storage at  $-80^{\circ}\text{C}$ .

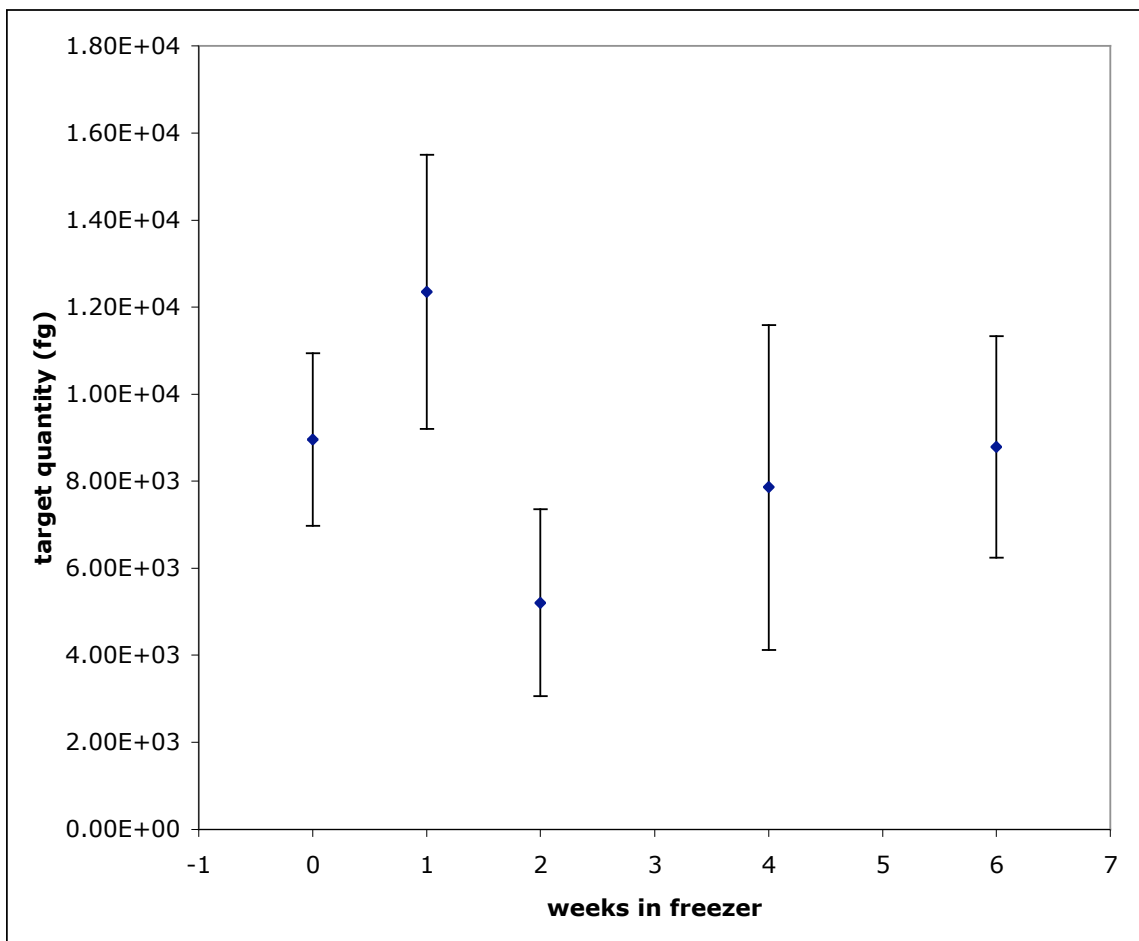
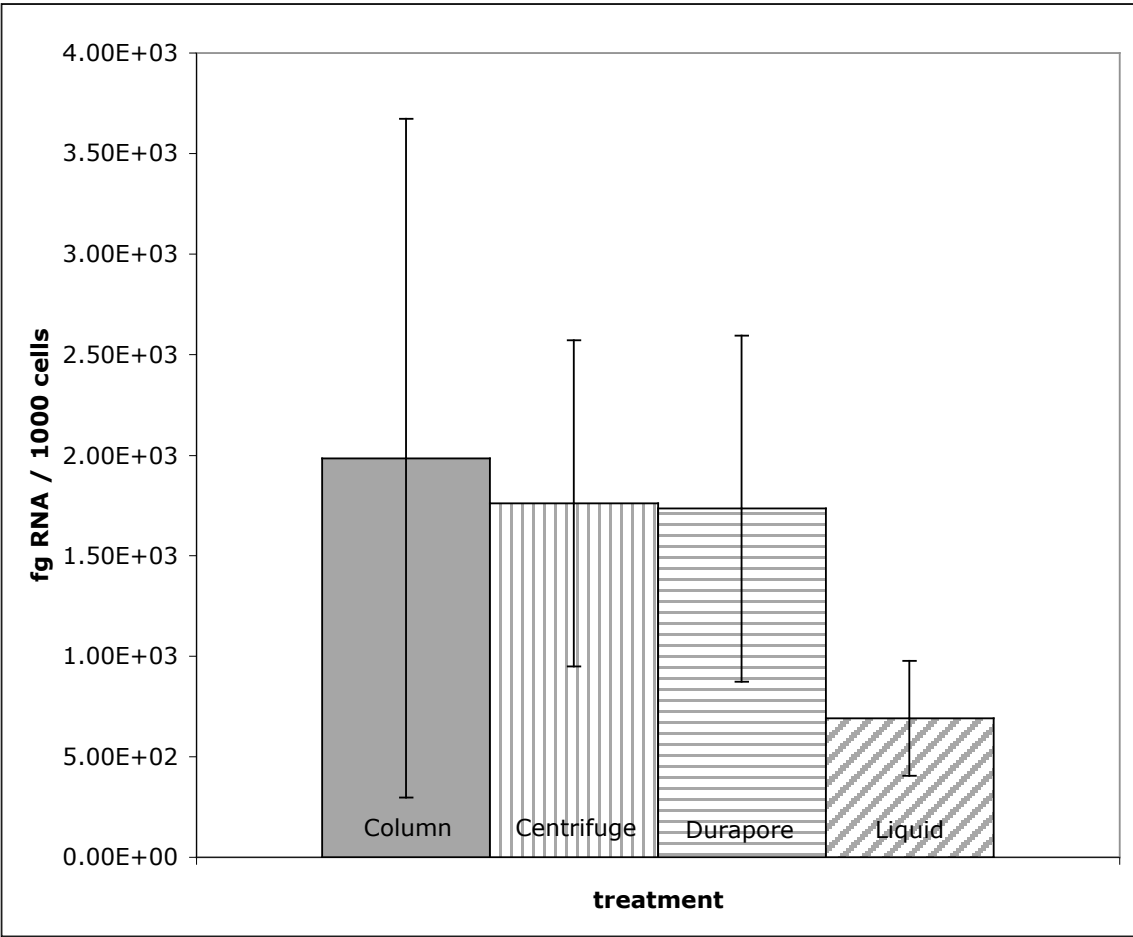




Figure 15: Detected *rbcL* RNA signal following alternative extraction methods. Plotted are the average values and the error bars represent the standard deviation (n=5 per treatment).



## Discussion

The real-time RT-PCR assay developed has performed well when applied to field samples. The assay was able to quantitate cellular abundance of *Karenia brevis* from natural populations without interference from other phytoplankton or other contamination inherent in natural marine samples for most of the samples analyzed. The assay was effective over a wide range of cell concentrations, ranging from below the direct microscope detection method limit of 300 cells liter<sup>-1</sup> to the highest sample analyzed at over 3X10<sup>5</sup> cells liter<sup>-1</sup>. Based on the cell standard curves, it would be possible to detect as many as 10<sup>4</sup> cells reaction<sup>-1</sup>, which corresponds to 10<sup>7</sup> cells liter<sup>-1</sup> if 20 ml is filtered, and 5  $\mu$ l of the 50  $\mu$ l column elution is used in the RT-PCR reaction. This assay, then, is able to detect and quantify the entire range of commonly seen *K. brevis* bloom concentrations.

The variation of *rbcL* transcript concentration in the cells of *K. brevis* do not appear to drastically or regularly change over a diel cycle, in contrast with carbon fixation. It is possible that RuBisCO activity in *K. brevis* is controlled at the translation or post translation level, or by changes in distribution within the chloroplast rather than at the transcription level as in *Gonyaulax* or *Lingulodinium* or as in synechococcus or a prymnesiophyte (47). There were some changes in the transcript signal at different times of the day and these changes did not appear to be a strictly regulated diurnal signal, as the changes did not occur at the same times in all of the diel experiments. These changes in

transcript levels could be a response to the cell's need for RuBisCO, natural fluctuations of a gene that is not strictly controlled at the transcriptional level, the inherent variability of the RT-PCR assay or the uneven distribution and fragility of cells in culture. Overall, changes between time points were relatively minor and often fell within the variability between replicates, as variability between replicates was large.

The variability of the *rbcL* transcript levels in *K. brevis* could lead to some error in the calculation of cell concentration from bloom samples by the real time RT-PCR assay, but the variation is minor and the assay is accurate enough to serve a useful purpose in the enumeration of *K. brevis* in the environment. Current guidelines use a binned system from categorization of blooms ranging from “not present” through “very high” (see Table 4). The ability of the real-time RT-PCR assay to reliably identify the category level of a bloom based on the system currently used by the FMRI is 54%. Without including the samples that were stored for a long period, this increases to 69%, indicating that long-term storage is not advisable when quantitative data are needed due to the increase of variability and potential loss of RNA as seen in Figure 14. Storage of samples should be kept to a minimum with samples processed as soon as possible. While storage at  $-80^{\circ}$  C of lysed culture samples demonstrated some level of stability, the RNA signal from bloom samples that were stored for a month or more was not detectable.

The developed RT-PCR assay, while effective for the detection and enumeration of *K. brevis*, appears to be a poor tool for analysis of gene expression due to both its variability and the variability of cell density in *K. brevis* culture, which may swamp the signal of transcriptional regulation. Its sensitivity is very high (as little as one copy of

target (49)) compared to that of other gene expression methods, such as dot-blotting and probing, but the signal of amplification becomes saturated at fairly low concentrations of target. Hybridization type assays, such as blotting and probing, have a much lower sensitivity, but are less likely to exhibit the variability of real time RT-PCR when sufficient target is present (67).

The method developed appears to be well suited to limited field use. The samples were stable at room temperature in Lysis buffer for at least 3 days, though the RNA target was not as stable in RLT. Therefore it would be preferable to use the RNA microprep extraction kit from Stratagene for the increased stability of the RNA target at room temperature as well as the decreased variability that was apparent in the room temperature stability experiment. If it is not possible to immediately extract and amplify the RNA from an environmental sample, it would be advisable to freeze them at  $-80^{\circ}\text{C}$ , rather than leave them at room temperature. The stability of the filtered samples was far greater when frozen at  $-80^{\circ}\text{C}$  than at room temperature. The samples that were frozen maintained most of their RNA throughout the experiment only losing RNA beginning at one month. Most of the RNA remained, however and even after 6 weeks, there was enough of the RNA to still detect, though quantitative representation of cell counts as inferred from real-time RT-PCR would be difficult due to the increased variability between samples. As the RNA began to degrade and the samples exhibited increased variability, it would be advisable to process samples that are to be quantitative within two weeks. If the samples have been in the freezer for more than 6 weeks, the assay may be unreliable as a quantitative method and should be only used as a presence/absence

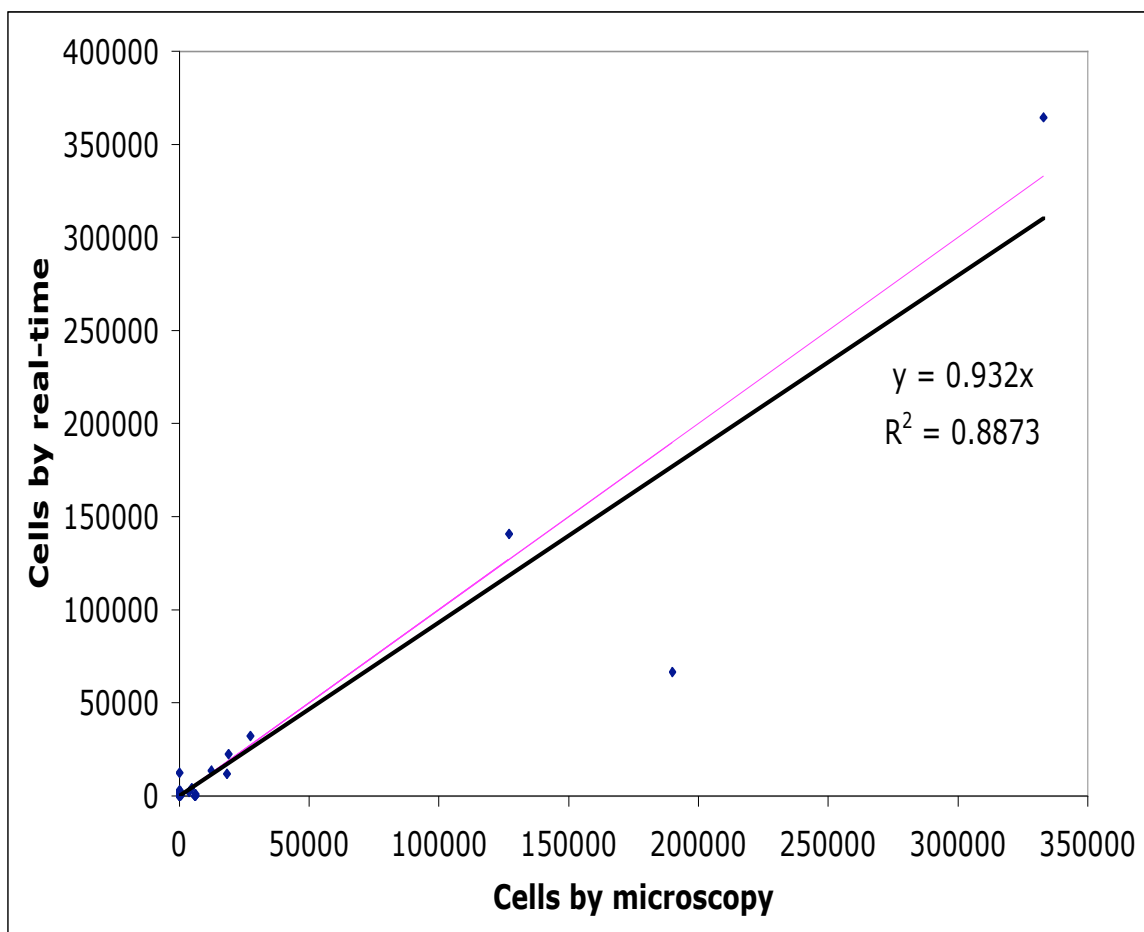
indicator, though even that may be too unreliable after samples had been frozen much longer than 6 weeks.

The bloom samples that were analyzed included some samples that had been frozen for over one month. According to the freezer stability experiment, these samples quantitative ability should have been reliable, however these samples inferred counts by real-time RT-PCR did not reflect the counts by microscopy. For comparison, those samples have been removed from the calculations in Figure 15(a) with the entire data set represented for comparison in Figure 15(b). The correlation between inferred counts by RT-PCR and microscopy counts has increased ( $r^2=.8873$ , vs.  $r^2=.8344$ ) and the slope of the linear regression trendline is closer to a slope of 1 ( $y=.932(x)$ , vs.  $y=.881(x)$ ) that would indicate perfect agreement. It is unclear why the bloom samples that had been stored at  $-80\text{ }^{\circ}\text{C}$  did not amplify in agreement with the microscopy counts, though there may have been compounds in the samples that inhibited the guanidine thiocyanate protection of the RNA. For this reason, samples from natural waters should not be frozen for extended periods if quantitative data are needed.

Experiments with the alternative extraction methods demonstrated distinct differences in target RNA content between the different methods. The increased variability of the column extraction method, despite its high yield of RNA, makes it a method that should be employed only if easier automation is required, as in a deployed system where the extra handling involved in a separate filtration or centrifugation step would be difficult to accomplish due to engineering difficulties and power requirements. Both the Durapore<sup>®</sup> filtration method and the centrifugation method, with their associated

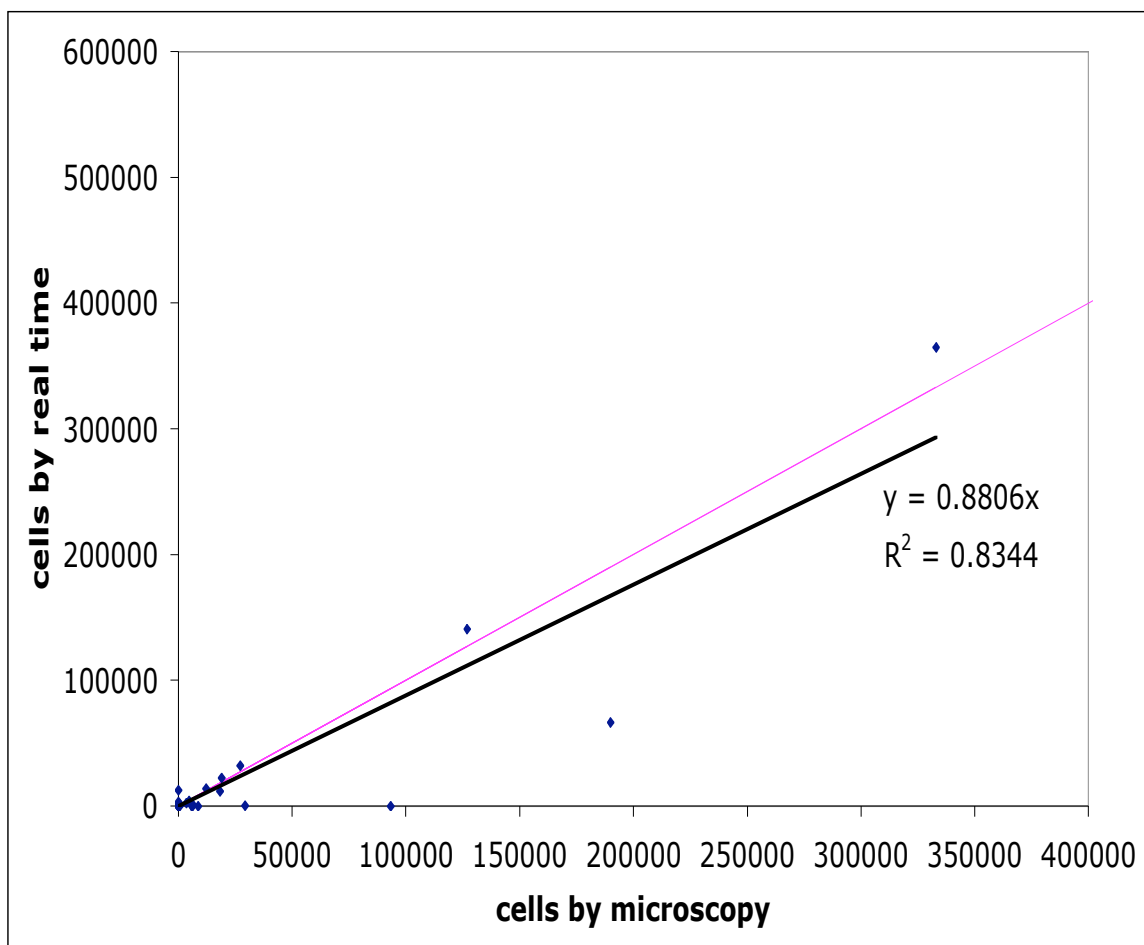
Figure 16: Cell counts by microscopy and the real-time RT-PCR assay. A) Cell counts by microscopy and the real-time RT-PCR assay not including samples that had been stored at  $-80^{\circ}\text{C}$  for over one month. B) Cell counts by microscopy and the real-time RT-PCR assay including samples that had been stored at  $-80^{\circ}\text{C}$  for over one month.

A)





B)



moderate variability and good RNA recovery are well suited to a procedure that would be used in the laboratory for quantitative evaluation of *K. brevis* abundance. Both methods have drawbacks inherent in the methods, however, when applied to natural bloom samples. For the Durapore® filtration method, filtration of samples, even the relatively small amount of 20 ml, can be difficult with some estuarine samples if there is a high load of detritus or suspended sediment. With the centrifugation method the volume needed to detect bloom populations may exceed the capacity of available centrifuges, though this is not a likely scenario in a relatively well-equipped laboratory. The liquid extraction protocol is the least likely to be useful, despite demonstrating the lowest variability. While it may be valuable for comparative studies using cultures, where extracted volumes are small, it would quickly become exceedingly cumbersome and expensive with higher volumes of sample to be extracted. For example, for 20 ml of a bloom sample to be extracted, 30 ml of lysis buffer would be required, as well as 50 ml of 70% ethanol, totaling 100 ml of lysate that must be loaded onto the extraction column, either using a vacuum manifold or 200 consecutive 30 second centrifugation steps. For these reasons, unless automation is a requirement it is recommended that either the Durapore® or centrifugation method be used for reproducible extraction and subsequent quantification of RNA from *K. brevis*.

These calculations indicate that when all of the recommendations made regarding processing of the samples, the assay can be very accurate in returning concentrations of *K. brevis* in the marine environment. Briefly, these recommendations include: process samples as soon as possible; if immediate processing is not possible, samples may be

frozen for a maximum of 3-4 weeks; if freezing is not possible, samples should be filtered, placed in Stratagene's lysis buffer and kept in the dark for a maximum of 3 days before freezing or processing. With these recommendations implemented, the assay performs very well, with a tendency to slightly overestimate cell concentrations in seawater samples. As a tool for managing and monitoring harmful algal blooms caused by *K. brevis* this trend of slight overestimation is preferable to underestimation and should not be cause for concern regarding the use of this method.

## References

1. **Aldrich, D. V.** 1962. Photoautotrophy in *Gymnodinium breve* Davis. *Science* **137**:988-&.
2. **Arkush, K. D., M. A. Miller, C. M. Leutenegger, I. A. Gardner, A. E. Packham, A. R. Heckerth, A. M. Tenter, B. C. Barr, and P. A. Conrad.** 2003. Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*). *International Journal for Parasitology* **33**:1087-1097.
3. **Baden, D. G., T. J. Mende, J. Walling, and D. R. Schultz.** 1984. Specific antibodies directed against toxins of *Ptychodiscus brevis* (Florida's red tide dinoflagellate). *Toxicon* **22**:783-789.
4. **Bjornland, T., F. T. Haxo, and S. Liaaen-Jensen.** 2003. Carotenoids of the Florida red tide dinoflagellate *Karenia brevis*. *Biochemical Systematics and Ecology* **31**:1147-1162.
5. **Bossart, G. D., D. G. Baden, R. Y. Ewing, B. Roberts, and S. D. Wright.** 1998. Brevetoxicosis in manatees (*Trichechus manatus latirostris*) from the 1996 epizootic: Gross, histologic, and immunohistochemical features. *Toxicologic Pathology* **26**:276-282.
6. **Carson, R. L.** 1951. *The Sea Around Us*. Oxford University Press, New York.
7. **Chen, Y. Z., J. Wu, A. Bharathi, and M. Manak.** 2003. Quantitative detection of SARS coronavirus using a TaqMan-based RT-PCR assay. *Clinical Chemistry* **49**:1965-1965.
8. **Culverhouse, P. F., R. Williams, B. Reguera, V. Herry, and S. Gonzalez-Gil.** 2003. Do experts make mistakes? A comparison of human and machine identification of dinoflagellates. *Marine Ecology-Progress Series* **247**:17-25.
9. **Dale, B., and C. M. Yentsch.** 1978. Red Tide and Paralytic Shellfish Poisoning. *Oceanus* **21**:41-49.

10. **Davis, C. C.** 1948. *Gymnodinium brevis* sp. nov., a cause of discolored water and animal mortality in the Gulf of Mexico. *Botanical Gazette* **109**:358-360.
11. **Fontaine, M., and E. Guillot.** 2003. Study of 18S rRNA and rDNA stability by real-time RT-PCR in heat-inactivated *Cryptosporidium parvum* oocysts. *Fems Microbiology Letters* **226**:237-243.
12. **Forrester, D. J., J. M. Gaskin, F. H. White, N. P. Thompson, J. A. Quick, G. E. Henderson, J. C. Woodard, and W. D. Robertson.** 1977. Epizootic of Waterfowl Associated with a Red Tide Episode in Florida. *Journal of Wildlife Diseases* **13**:160-167.
13. **Fratamico, P. M.** 2003. Comparison of culture, polymerase chain reaction (PCR), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for detection of *Salmonella* spp. in naturally-contaminated ground chicken, ground turkey, and ground beef. *Molecular and Cellular Probes* **17**:215-221.
14. **Fuhrman, J. A., D. E. Comeau, Å. Hagstrom, and A. M. Chan.** 1998. Extraction from natural planktonic microorganisms of DNA suitable for molecular biological studies. *Applied and Environmental Microbiology* **54**:1426-1429.
15. **Geraci, J. R.** 1989. Clinical investigations of the 1987-1988 mass mortality of bottlenose dolphins along the U.S. central and south Atlantic coast. Ontario Veterinary College.
16. **Gibson, J. L., and F. R. Tabita.** 1977. Different molecular forms of D-ribulose-1,5-bisphosphate carboxylase from *Rhodospseudomonas sphaeroides*. *Journal of Biological Chemistry* **252**:943-949.
17. **Godhe, A., S. K. Otta, A. S. Rehnstam-Holm, I. Karunasagar, and I. Karunasagar.** 2001. Polymerase chain reaction in detection of *Gymnodinium mikimotoi* and *Alexandrium minutum* in field samples from southwest India. *Marine Biotechnology* **3**:152-162.
18. **Guillard, R. R., and J. H. Ryther.** 1962. Studies of marine planktonic diatoms. 1. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Canadian Journal of Microbiology* **8**:229-239.
19. **Guillou, L., E. Nezan, V. Cueff, E. E. L. Denn, M. A. Cambon-Bonavita, P. Gentien, and G. Barbier.** 2002. Genetic diversity and molecular detection of three toxic dinoflagellate genera (*Alexandrium*, *Dinophysis*, and *Karenia*) from French coasts. *Protist* **153**:223-238.

20. **Gunther, G., R. H. Williams, C. C. Davis, and F. G. W. Smith.** 1947. Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida. *Florida Ecological Monographs* **18**:311-324.
21. **Guy, R. A., P. Payment, U. J. Krull, and P. A. Horgen.** 2003. Real-time PCR for quantification of *Giardia* and *Cryptosporidium* in environmental water samples and sewage. *Applied and Environmental Microbiology* **69**:5178-5185.
22. **Hallegraeff, G. M.** 1993. A Review of Harmful Algal Blooms and Their Apparent Global Increase. *Phycologia* **32**:79-99.
23. **Heid, C. A., J. Stevens, K. J. Livak, and P. M. Williams.** 1996. Real time quantitative PCR. *Genome Research* **6**:986-994.
24. **Heil, C. A.** 1986. Vertical Migration of *Ptychodiscus brevis* (Davis) Steidinger. Master of Science. University of South Florida, St. Petersburg.
25. **Hersh, S. H.** 1998. Why the dolphins die. *Sea Frontiers* **July-August**:246-248.
26. **Hird, H., J. Lloyd, R. Goodier, J. Brown, and P. Reece.** 2003. Detection of peanut using real-time polymerase chain reaction. *European Food Research and Technology* **217**:265-268.
27. **Hoagland, P., D. M. Anderson, Y. Kaoru, and A. W. White.** 2002. The economic effects of harmful algal blooms in the United States: Estimates, assessment issues, and information needs. *Estuaries* **25**:819-837.
28. **Ibrahim, M. S., D. A. Kulesh, S. S. Saleh, I. K. Damon, J. J. Esposito, A. L. Schmaljohn, and P. B. Jahrling.** 2003. Real-time PCR assay to detect smallpox virus. *Journal of Clinical Microbiology* **41**:3835-3839.
29. **Inokuchi, M., I. Ninomiya, K. Tsugawa, I. Terada, and K. Miwa.** 2003. Quantitative evaluation of metastases in axillary lymph nodes of breast cancer. *British Journal of Cancer* **89**:1750-1756.
30. **Ishida, K., and B. R. Green.** 2002. Second- and third-hand chloroplasts in dinoflagellates: Phylogeny of oxygen-evolving enhancer 1 (PsbO) protein reveals replacement of a nuclear-encoded plastid gene by that of a haptophyte tertiary endosymbiont. *Proceedings of the National Academy of Sciences of the United States of America* **99**:9294-9299.
31. **Kamykowski, D., E. J. Milligan, and R. E. Reed.** 1998. Biochemical relationships with the orientation of the autotrophic dinoflagellate *Gymnodinium breve* under nutrient replete conditions. *Marine Ecology-Progress Series* **167**:105-117.

32. **Kamykowski, D., E. J. Milligan, and R. E. Reed.** 1998. Relationships between geotaxis/phototaxis and diel vertical migration in autotrophic dinoflagellates. *Journal of Plankton Research* **20**:1781-1796.
33. **Knight, S., I. Andersson, and C. I. Branden.** 1990. Crystallographic analysis of ribulose 1,5-bisphosphate carboxylase from spinach at 2.4 Å resolution - subunit interactions and active-site. *Journal of Molecular Biology* **215**:113-160.
34. **Kreuder, C., J. A. K. Mazet, G. D. Bossart, T. E. Carpenter, M. Holyoak, M. S. Elie, and S. D. Wright.** 2002. Clinicopathologic features of suspected brevetoxicosis in double-crested cormorants (*Phalacrocorax auritus*) along the Florida Gulf Coast. *Journal of Zoo and Wildlife Medicine* **33**:8-15.
35. **Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei.** 2001. MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**:1244-1245.
36. **Layne, J. N.** 1965. Observations on marine mammals in Florida waters. *Bulletin of the Florida State Museum, Biological Sciences* **9**:131-181.
37. **Lee, M. S., G. W. Qin, K. Nakanishi, and M. G. Zagorski.** 1989. Biosynthetic-Studies of Brevetoxins, Potent Neurotoxins Produced by the Dinoflagellate *Gymnodinium breve*. *Journal of the American Chemical Society* **111**:6234-6241.
38. **Millie, D. F., O. M. Schofield, G. J. Kirkpatrick, G. Johnsen, P. A. Tester, and B. T. Vinyard.** 1997. Detection of harmful algal blooms using photopigments and absorption signatures: A case study of the Florida red tide dinoflagellate, *Gymnodinium breve*. *Limnology and Oceanography* **42**:1240-1251.
39. **Moreira, D., and H. Philippe.** 2001. Sure facts and open questions about the origin and evolution of photosynthetic plastids. *Research in Microbiology* **152**:771-780.
40. **Morse, D.** 1995. A nuclear-encoded form-II RuBisCO in dinoflagellates. *Science* **269**:17.
41. **Naar, J., A. Bourdelais, C. Tomas, J. Kubanek, P. L. Whitney, L. Flewelling, K. Steidinger, J. Lancaster, and D. G. Baden.** 2002. A competitive ELISA to detect brevetoxins from *Karenia brevis* (formerly *Gymnodinium breve*) in seawater, shellfish, and mammalian body fluid. *Environmental Health Perspectives* **110**:179-185.

42. **Nassoury, N., L. Fritz, and D. Morse.** 2001. Circadian changes in ribulose-1,5-bisphosphate carboxylase/oxygenase distribution inside individual chloroplasts can account for the rhythm in dinoflagellate carbon fixation. *Plant Cell* **13**:923-934.
43. **Oberst, R. D., M. P. Hays, L. K. Bohra, R. K. Phebus, and J. M. Sargeant.** 2003. Detection of *Escherichia coli* O157 : H7 in cattle feces using a polymerase chain reaction-based fluorogenic 5' nuclease (TaqMan (R)) detection assay after secondary enrichment. *Journal of Veterinary Diagnostic Investigation* **15**:543-552.
44. **Okamoto, O. K., and J. W. Hastings.** 2003. Novel dinoflagellate clock-related genes identified through microarray analysis. *Journal of Phycology* **39**:519-526.
45. **Ornoldsdottir, E. B., J. L. Pinckney, and P. A. Tester.** 2003. Quantification of the relative abundance of the toxic dinoflagellate, *Karenia brevis* (Dinophyta), using unique photopigments. *Journal of Phycology* **39**:449-457.
46. **Oshea, T. J., G. B. Rathbun, R. K. Bonde, C. D. Buergelt, and D. K. Odell.** 1991. An Epizootic of Florida Manatees Associated with a Dinoflagellate Bloom. *Marine Mammal Science* **7**:165-179.
47. **Paul, J. H., J. B. Kang, and F. R. Tabita.** 2000. Diel patterns of regulation of *rbcl* transcription in a cyanobacterium and a prymnesiophyte. *Marine Biotechnology* **2**:429-436.
48. **Pierce, R. H., M. S. Henry, P. C. Blum, J. Lyons, Y. S. Cheng, D. Yazzie, and Y. Zhou.** 2003. Brevetoxin concentrations in marine aerosol: Human exposure levels during a *Karenia brevis* harmful algal bloom. *Bulletin of Environmental Contamination and Toxicology* **70**:161-165.
49. **Rauter, H., M. Overeijnder, D. Doornbosch, and T. Schalkhammer.** 2003. DegP and related genes as stress-markers for *E. coli* viability - Ultra-sensitive RT-real-time PCR. *Monatshefte Fur Chemie* **134**:1489-1498.
50. **Robledo, R., W. Beggs, and P. Bender.** 2003. A simple and cost-effective method for rapid genotyping of insertion/deletion polymorphisms. *Genomics* **82**:580-582.
51. **Ryu, C., K. Lee, C. Yoo, W. K. Seong, and H. B. Oh.** 2003. Sensitive and rapid quantitative detection of anthrax spores isolated from soil samples by real-time PCR. *Microbiology and Immunology* **47**:693-699.



52. **Sieracki, C. K., M. E. Sieracki, and C. S. Yentsch.** 1998. An imaging-in-flow system for automated analysis of marine microplankton. *Marine Ecology-Progress Series* **168**:285-296.
53. **Spreitzer, R. J., and M. E. Salvucci.** 2002. Rubisco: Structure, regulatory interactions, and possibilities for a better enzyme. *Annual Review of Plant Biology* **53**:449-475.
54. **Steidinger, K., and R. M. Ingle.** 1972. Observations on 1971 Summer Red Tide in Tampa Bay, Florida. *Environmental Letters* **3**:271-278.
55. **Steidinger, K. A., and E. A. Joyce, Jr.** 1973. Florida Red Tides. State of Florida Department of Natural Resources Education Series **17**:1-26.
56. **Stumpf, R. P.** 2001. Applications of satellite ocean color sensors for monitoring and predicting harmful algal blooms. *Human and Ecological Risk Assessment* **7**:1363-U15.
57. **Tabita, F. R.** 1999. Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: a different perspective. *Photosynthesis Research* **60**:1-28.
58. **Tabita, F. R., and B. A. McFadden.** 1974. One-step isolation of microbial ribulose-1,5-diphosphate carboxylase. *Archives of Microbiology* **99**:231-240.
59. **Takishita, K., K. Nakano, and A. Uchida.** 2000. Origin of the plastid in the anomalously pigmented dinoflagellate *Gymnodinium mikimotoi* (Gymnodiniales, Dinophyta) as inferred from phylogenetic analysis based on the gene encoding the large subunit of form I-type RuBisCO. *Phycological Research* **48**:85-89.
60. **Tengs, T., H. A. Bowers, A. P. Ziman, and D. K. Stoecker.** 2001. Genetic polymorphism in *Gymnodinium galatheanum* chloroplast DNA sequences and development of a molecular detection assay. *Molecular Ecology* **10**:515-523.
61. **Tester, P. A., and K. A. Steidinger.** 1997. *Gymnodinium breve* red tide blooms: Initiation, transport, and consequences of surface circulation. *Limnology and Oceanography* **42**:1039-1051.
62. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. Clustal-W - Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Research* **22**:4673-4680.
63. **Trainer, V. L., and D. G. Baden.** 1999. High affinity binding of red tide neurotoxins to marine mammal brain. *Aquatic Toxicology* **46**:139-148.

64. **Tyrrell, J. V., P. R. Bergquist, P. L. Bergquist, and C. A. Scholin.** 2001. Detection and enumeration of *Heterosigma akashiwo* and *Fibrocapsa japonica* (Raphidophyceae) using rRNA-targeted oligonucleotide probes. *Phycologia* **40**:457-467.
65. **Ulstrup, K. E., and M. J. H. Van Oppen.** 2003. Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Molecular Ecology* **12**:3477-3484.
66. **Van Dolah, F. M., and T. A. Leighfield.** 1999. Diel phasing of the cell-cycle in the Florida red tide dinoflagellate, *Gymnodinium breve*. *Journal of Phycology* **35**:1404-1411.
67. **Wawrik, B., J. H. Paul, and F. R. Tabita.** 2002. Real-time PCR quantification of rbcL (ribulose-1,5-bisphosphate carboxylase/oxygenase) mRNA in diatoms and pelagophytes. *Applied and Environmental Microbiology* **68**:3771-3779.
68. **Whitney, S. M., D. C. Shaw, and D. Yellowlees.** 1995. Evidence That Some Dinoflagellates Contain a Ribulose-1,5-Bisphosphate Carboxylase Oxygenase Related to That of the Alpha-Proteobacteria. *Proceedings of the Royal Society of London Series B-Biological Sciences* **259**:271-275.
69. **Wilson, W. B.** 1967. Forms of Dinoflagellate *Gymnodinium breve* Davis in Cultures. *Contributions in Marine Science* **12**:120-&.
70. **Zen, H., K. I. Nakashiro, S. Shintani, T. Sumida, T. Aramoto, and H. Hamakawa.** 2003. Detection of circulating cancer cells in human oral squamous cell carcinoma. *International Journal of Oncology* **23**:605-610.
71. **Zhang, H., and S. Lin.** 2002. Detection and quantification of *Pfiesteria piscicida* by using the mitochondrial cytochrome *b* gene. *Applied and Environmental Microbiology* **68**:989-994.

## **Appendices**

## Appendix 1

Media Recipes:

### F/2 medium

To 950 ml of filtered seawater, add:

Quantity	Compound	Stock Solution (in deionized water)
1.0 ml	NaNO <sub>3</sub>	75.0 g L <sup>-1</sup>
1.0 ml	NaH <sub>2</sub> PO <sub>4</sub> • H <sub>2</sub> O	5.0 g L <sup>-1</sup>
1.0 ml	Na <sub>2</sub> SiO <sub>3</sub> • 9H <sub>2</sub> O	30.0 g L <sup>-1</sup>
1.0 ml	F/2 Trace Metal Solution	(See Recipe Below)
0.5 ml	F/2 Vitamin Solution	(See Recipe Below)

Adjust final volume to 1.0 L with filtered seawater, autoclave

### Trace Metal Solution

To 950 ml deionized water, add:

Quantity	Compound	Stock Solution (in deionized water)
3.15 g	FeCl <sub>3</sub> • 6H <sub>2</sub> O	--
4.36 g	Na <sub>2</sub> EDTA • 2H <sub>2</sub> O	--
1.0 ml	CuSO <sub>4</sub> • 5H <sub>2</sub> O	9.8 g L <sup>-1</sup>
1.0 ml	Na <sub>2</sub> MoO <sub>4</sub> • 2H <sub>2</sub> O	6.3 g L <sup>-1</sup>
1.0 ml	ZnSO <sub>4</sub> • 7H <sub>2</sub> O	22.0 g L <sup>-1</sup>
1.0 ml	CoCl <sub>2</sub> • 6H <sub>2</sub> O	10.0 g L <sup>-1</sup>
1.0 ml	MnCl <sub>2</sub> • 4H <sub>2</sub> O	180.0 g L <sup>-1</sup>

Adjust final volume to 1.0 L, autoclave.

## Appendix 1 (Continued)

### Vitamin Solution

To 950 ml deionized water, add:

Quantity	Compound	Stock Solution (in deionized water)
1.0 ml	Vitamin B12 (Cyanocobalamin)	1.0 g L <sup>-1</sup>
10.0 ml	Biotin	0.1 g L <sup>-1</sup>
200 mg	Thiamine HCl	--

Adjust final volume to 1.0 L with deionized water. Filter sterilize into plastic vials and store in refrigerator.

\*Note that f/2 media is high in Silicon and should only be used for culture of diatoms. For cultures of non-diatoms, use f/2-Si (omit the Na<sub>2</sub>SiO<sub>3</sub> 9H<sub>2</sub>O )

### 2XYT

to 950 ml deionized water, add:

16.0 g Tryptone

10.0 g Yeast Extract

5.0 g NaCl

adjust volume to 1.0 L with deionized water and autoclave.

For agar plates, add 15.0 g Bacto Agar, boil to dissolve, then autoclave.

If antibiotics are needed, add 1.0 ml Kanamycin (50 mg/ml) and/or 1.0 ml Ampicillin (50 mg/ml).

### SN medium

Quantity	Compound	Stock solution
2.5 ml	NaNO <sub>3</sub>	300.0 g L <sup>-1</sup>
2.6 ml	K <sub>2</sub> HPO <sub>4</sub>	6.1 g L <sup>-1</sup>
5.6 ml	Na <sub>2</sub> EDTA•2H <sub>2</sub> O	1.0 g L <sup>-1</sup>
2.6 ml	Na <sub>2</sub> CO <sub>3</sub>	4.0 g L <sup>-1</sup>
1.0 ml	Vitamin B12	1.0 mg L <sup>-1</sup>
1.0 ml	Trace metal solution	(see below)

Add ingredients aseptically to 1 L autoclaved 75% seawater in a Teflon bottle.

## Appendix 1 (Continued)

### Trace Metal Solution

Quantity	Compound
6.25 g	Citric acid • H <sub>2</sub> O
6.0 g	Ferric Ammonium Citrate
1.4 g	MnCl <sub>2</sub> • 4H <sub>2</sub> O
0.39 g	Na <sub>2</sub> MoO <sub>4</sub> • 2H <sub>2</sub> O
0.025 g	Co(NO <sub>3</sub> ) <sub>2</sub> • 6H <sub>2</sub> O
0.222 g	ZnSO <sub>4</sub> • 7H <sub>2</sub> O

Dissolve each metal compound individually in 100 ml deionized water. Combine and mix the solutions, then bring final volume to 1.0 L with deionized water.

## Appendix 1 (Continued)

Strains cultured and their media requirements:

Organism	strain	media required	growth temperature
<b>Dinoflagellates</b>			
<i>K. brevis</i>	various	f/2 -Si	22° C
<i>K. mikimotoi</i>	CCMP430	f/2 -Si	16° C
<i>Amphidinium carterae</i>	CCMP1314	f/2 -Si	22° C
<i>Akashiwo sanguinea</i>	CCMP1321	f/2 -Si	22° C
<i>Alexandrium tamarense</i>	CCMP1493	f/2 -Si	22° C
<i>Glenodinium foliacrum</i>	N/A	f/2 -Si	22° C
<i>Gymnodinium catenatum</i>	CCMP1937	f/2 -Si	22° C
<i>Gyrodinium</i> sp.	N/A	f/2 -Si	22° C
<i>Kryptoperidinium foliaceum</i>	N/A	f/2 -Si	22° C
<i>Lingulodinium polyedra</i>	CCMP1738	f/2 -Si	22° C
<i>Prorocentrum micans</i>	N/A	f/2 -Si	22° C
<i>Scrippsiella trochoidea</i>	N/A	f/2 -Si	22° C
<i>Scrippsiella precaria</i>	N/A	f/2 -Si	22° C
<b>Diatom</b>			
<i>Phaeodactylum tricornutum</i>	CCMP1327	f/2	22° C
<b>Raphidophyte</b>			
<i>Heterosigma akashiwo</i>	N/A	f/2 -Si	22° C
<b>Praesinophytes</b>			
<i>Tetraselmis</i> sp.	850001	f/2 -Si	22° C
<i>Tetraselmis</i> sp.	CCMP961	f/2 -Si	22° C
Unidentified sp.	CCMP1536	f/2 -Si	22° C
<b>Prymnesiophytes</b>			
<i>Isochrysis</i> sp.	3C		
<i>Pavlova lutheri</i>	CCMP1325	f/2 -Si	16° C
<i>Prymnesium parvum</i>	N/A	f/2 -Si	22° C
<b>Chlorophyte</b>			
<i>Clamydomonas euryale</i>	CCMP219	f/2 -Si + NH4	22° C
<b>Cyanophytes</b>			
<i>Synechococcus</i> sp.	CCMP836	SN	22° C
<i>Synechococcus</i> sp.	WH7803	SN	22° C
<b>Trebouxiophyte</b>			
<i>Chlorella autotrophica</i>	CCMP243	f/2	16° C
<b>Coscinodiscophyte</b>			
<i>Thalassiosira pseudonana</i>	CCMP 1335	f/2	16° C

## Appendix 2

### Multiple sequence alignment of *rbcL* DNA sequences used in phylogenetic analysis

```
Emiliana_huxleyi: GGTGGTCTAGACTTCCTTAAGGATGATGAGAACATTAACTCACAGCCATT
ST8CH26_clone: -----C---CATT
Isochrysis: GGTGGTCTAGACTTCCTTAAGGATGATGAGAACATTAACTCACACCATT
Calcidiscus: GGTGGTCTAGACTTCCTTAAGGATGATGAGAACATTAACTCACACCATT
Cruciplacolithus: GGTGGTCTAGACTTCCTTAAGGATGATGAGAACATTAACTCACAGCCGTT
Pleurochrysis: GGTGGTCTAGATTTCTTAAGGACGACGAGAACATTAACTCACACCATT
Pavlova: GGTGGTCTGGACTTCCTTAAGGATGACGAAAACATCAACTCACACCATT
Exanthemachrysis: GGTGGTCTAGACTTCCTTAAGGATGACGAGAACATTAACTCACACCATT
Pyrenomonas: GGCGGTCTTGACTTCCTTAAGGATGATGAAAACATCAACTCTCAACCATT
Chroomonas: GGTGGTTAGACTTCCTTAAAGATGATGAAAACATTAACTCTCAACCATT
Rhodella: GGTGGATTAGACTTCCTTAAAGGATGATGAAAACATTAACTCTCAACCATT
Dictyocha: GGTGGTCTTGATTTCTTAAAGATGATGAGAATATTAACTCACAGCGTT
ST6CH33_clone: -----C---CATT
Heterosigma: ATTTTTTCTTAGACTGTACACTAACGTACAAAATTAATAAGGAGGAATA
Chattonella: GGTGGTCTTGACTTCCTTAAAGATGATGAGAACATTAACTCTCAACCATT
ST4CH31_clone: -----C---CATT
ST1CH4_clone: -----CAACCATT
ST3CH27_clone: -----CAAGCATT
Ectocarpus: GGTGGTCTTGACTTCCTTAAAGATGATGAAAATATTAATTCACAACCATT
Xanthonema: GGTGGTCTTGACTTCCTTAAAGATGATGAAAACATTAACTCTCAACCATT
Vaucheria: GGTGGTTTAGATTTCTTAAAGATGATGAGAATATTAATTCCTCAACCATT
Heterococcus: GATATGCGAATTCCTTTTGCTTACCTAAAAACATTCGAAGTCTGCAAC
Aureombra: GGTGGTTAGACTTTTTAAAGGATGATGAAAACATTAACTCGCAACCATT
Aureococcus: GGTGGTTAGACTTCCTTAAAGGATGATGAGAACATTAACTCACACCATT
Pelagococcus: GGTGGTTAGACTTCCTTAAAGGATGACGAGAACATTAACTCACACCATT
Cylindrotheca: GGTGGTTAGACTTTTTAAAGATGATGAAAACATTAACTCACACCATT
Phaeodactylum: GGTGGTTAGACTTCCTTAAAGATGATGAGAATATTAACTCACACCCTT
ST4CH14_clone: -----C---CATT
ST6CH2_clone: -----C---CATT
Rhizosolenia: GGTGGTTAGACTTCCTTAAAGATGATGAAAACATTAACTCACACCATT
Skeletonema: GGTGGTTAGACTTCCTTAAAGGATGATGAAAATATTAACTCTCAACCATT
Thalassiosira: GGTGGTTAGACTTCCTTAAAGATGATGAAAACATTAACTCTCAACCATT
Detonula: CGTCGTGTATGCCAGTAGCTTCTGGTGGTATCCACTGTGGTCAAATGCA
Chilomonas: GGTGGCTTGACTTCCTTAAGGATGATGAAAACATTAATTCCTCAGCCTTT
Mazzaella_membranacea: GGTGGTTAGACTTCCTTAAAGATGATGAGAATATTAATTCCTCAGCCTTT
Karenia_brevis: GGGGGATTAGATTTCTTAAAGATGATGAAAATATTAATTCGCAGCCTTT
Jaxonville_C3a: -----GATGATGAAAATATTAACTCGCAGCCTTT
Mexico_Beach_C5: -----GATGATGAAAATATTAACTCGCAGCCTTT
Piney_Island_A9: -----GATGATGAAAATATTAACTCGCAGCCTTT
Charlotte_Harbor_C2: -----GATGATGAAAATATTAACTCGCAGCCTTT
Charlotte_Harbor_A2: -----TATGATGAAAATATTAACTCGCAGCCTTT
Piney_Island_B4: -----GATGATGAGAATATTAACTCGCAGCCTTT
Mexico_Beach_B3: -----GATGAGAACATTAACTCGCAGCCTTT
Appalachicola_C6: -----GATGATGAGAATATTAACTCGCAGCCTTT
Karenia_mikimotoi_1: -----GATGATGAGAATATTAACTCACACCATT
Karenia_mikimotoi: GGAGGCTTGATTTCTTAAAGGATGATGAGAACATTAACTCACACCATT
Karlodinium: GGCGGTTTGATTTCTTAAAGATGATGAAAATATCAATTCCTCAACCATT

Amphidinium: CTTGCTAACATGTCAAGCGAAGCCCTGTGCGACGGTCTTCACCATAGGTGC
ST2SY33_clone: GGTGGTCTCGATTTAACGAAGGATGACGAGAATATAAATCTCAGCCATT
Prochlorococcus: GGCGGTCTTGATTTAACGAAGGATGATGAGAATATTAATTCCTCAACCATT
```



## Appendix 2 (Continued)

Thiobacillus: CTACGTCAAGACCTGCGGTGGGCCACCCACGGGATTCAGGTTCGAACGCG  
 Synechococcus\_sp: GGTGGTTTGGACTTCACCAAAGATGACGAAAACATCAACTCCCAGCCTTT  
 ST2SY26\_clone: GGTGTTTTGNACTTCACCAAAGACGACGAGAATCAACTNTCAGCCCTT  
 Synechococcus: GTTCATCAAGAGCTGCTACGGCCCGCCGAACGGCATCCAGGTTCGAGCGCG  
 Nitrobacter: TTACGTGAAGACCTGCGGCGGCCCTCCGCATGGTATCCAGGTTCGAGCGCG  
 Synechocystis: GGTGGTTTGGACTTCACCAAAGACGACGAAAACATCAACTCCCAGCCTTT  
 Synechococcus\_PCC7002: GGTGGTCTTGACTTCACCAAAGATGACGAAAACATCAACTCTCAGCCTTT  
 ST8SY15\_clone: GGAGGTCTAGACTTCACCAAAGATGACGAAAATATTAACTCTCAACCTTT  
 Trichodesmium: GGAGGTCTAGACTTCACCAAAGATGACGAAAATATTAACTCTCAACCTTT  
 ST5SY7\_clone: GGTGGTTTAGATTTTACTAAGGATGACGAGAATCAACTCTCAACCTTT  
 ST2SY2\_clone: -----GATGAGAACGTAAACTCACAACCTTT  
 ST6SY8\_clone: GGTGGTCTTGACTTCACCTAAGGATGATGAGAACGTAAACTCCCAGCCTTT  
 Volvox\_carteri: GATCTTCGTATTCACCTGCTTACGTTAAAACATTCCAAGGTCCACCACA  
 Spinacia: GGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAACCTCCCAGCGTT  
 Asparagus: GGTGGGCTTGATTTTACCAAAGATGATGAAAACGTGAACCTCACAACCTTT  
 Thalassia: GATTTGCGAATTCCTTCTTCCAAAACCTTCCAAGGTCCACCTCA  
 Prymnesium: GGTGGTCTTGATTTCTTAAGGATGATGAGAACATTAACTCTCAACCATT  
 Chrysochromulina\_sp: GGTGGTCTTGACTTCCTAAAGGATGATGAGAACATTAACTCTCAGCCATT  
 Imantionia: GGTGGTCTTGACTTCCTAAAGGATGACGAGAACATTAACTCGCAGCCTTT  
 ST1CH3\_clone: -----C-----CATT  
 Helicosphaera: GGAGGTCTAGATTTCTTAAGGATGATGAGAACATCAACTCACAACCTTT  
  
 Emiliana\_huxleyi: CATGCGTTACCGTGAGCGTTTCTTTACTCAATGGAAGGTGTTAACCATG  
 ST8CH26\_clone: CATGCGTTACAGAGAGCGTTTCTTATACTCAATGNAAGGTGTTAACCCAG  
 Isochrysis: TATGCGTTACCGTGAGCGTTTCTTTACTCAATGGAAGGTGTTAACCAACG  
 Calcidiscus: TATGCGTTACAGAGAGCGTTTCTTTACTCAATGGAAGGTGTTAACCCAG  
 Crucioplacolithus: CATGCGTTACAGAGAGCGTTTCTTTACTCAATGGAAGGTGTTAACCCAG  
 Pleurochrysis: CATGCGTTACCGCGAGCGTTTCTTTACTCAATGGAAGGTGTTAACCCATG  
 Pavlova: TATGCGTTGGAGAGAGCGTTTCTTATTCTCGATGGAAGGTGTTAACCCGTG  
 Exanthemachrysis: CATGCGTTGGAGAGAGCGTTTCTTATTCTCAATGGAAGGTGTTAACCCGTG  
 Pyrenomonas: CATGAGATGGAAAGAGCGTTTCTTATTCTCGATGGAAGGTGTTAACAGAG  
 Chromonas: CATGAGATGGAAAGAGCGTTTCTTATTCTCGATGGAAGGTGTTAACCCGTG  
 Rhodella: TATGCGTTACAGAGAGCGTTTCTTATTCTCTATGGAAGGTGTTAACCCGTG  
 Dictyocha: CATGCGTTGGAGAGAACGTTTCTTTACTGTCAAGAAGGTATTAACCCGTG  
 ST6CH33\_clone: CATGCGTTGGAGAGAGCGTTTCTTATACGTAATGGAAGGTGTTAACCCGTG  
 Heterosigma: CATGTCTAACAAACGTATACGAACGTAAACCGAATCAAAAATGATCGTTATG  
 Chattonella: CATGCGTTGGAGAGAGCGTTTCTTTACTGTATGGAAGGTATTAATCGTG  
 ST4CH31\_clone: CATGCGTTGGAGAGAACGTTTCTTATACTGTATGGAAGGTATTAATCGTG  
 ST1CH4\_clone: CATGAGATGGCGTGAAGATACTTATACGTTATGGAAGGAATTAACAAAG  
 ST3CH27\_clone: CATGAGATGGAGAGAAAGATTCTTACTGTAATGGAAGGTGTTAACCCGTG  
 Ectocarpus: TATGCGTTGGAAAGAACGTTTCTTATACTGTATGGAAGGTGTTAACCCGTG  
 Xanthonema: CATGCGTTGGAGAGAACGTTTCTTTACTGTATGGAAGGTATTAACCCGTG  
 Vaucheria: TATGCGTTGGAGAGAGCGTTTCTTTACTGTATGGAAGGTGTTAATCGTG  
 Heterococcus: TGGTGTATTGTAGAAAGAGAAAGAAATGGACA---AATTCGGTCGCCCTT  
 Aureoumbra: CATGCGTTGGCGTGAGCGTTTCTTATACTGTATCGAAGGTATTAATCGTG  
 Aureococcus: CATGCGTTGGCGTGAGCGTTTCTTACTGTGTAGAGGGTATCAACCCGTG  
 Pelagococcus: CATGCGTTGGCGTGAGCGTTTCTTATACTGTGTAGAAGGTATTAACCCGTG  
 Cylindrotheca: TATGCGTTGGAGAGAACGTTTCTTAAACTGTATGGAAGGTATTAACCCGTG  
 Phaeodactylum: CATGCGTTGGAGAGAACGTTTCTTATACTGTATGGAAGGTATTAATCGCG  
 ST4CH14\_clone: CATGCGTTGGAGAGAGCGTTTCTTAAACTGTATGGAAGGTATCAACCCGTG  
 ST6CH2\_clone: CATGCGTTGGAGAGAGCGTTTCTTATACTGTATGGAAGGTATTAACCCGTG  
 Rhizosolenia: CATGCGTTGGAGAGAACGTTTCTTATACTGTATGGAAGGTATTAACCCGTG  
 Skeletonema: CATGCGTTGGAGAGAACGTTTCTTAAACTGTATGGAAGGTATTAACCCGTG  
 Thalassiosira: TATGCGTTGGAGAGAACGTTTCTTAAACTGTTTAGAAGGTATCAACCCGTG

## Appendix 2 (Continued)

Detonula: TCAATTAATTCATTACTTAGGTGATGATGTTGTATTACAATTCGGTGGTG  
 Chilomonas: TATGAGATGGAGAGAACGCTTCCTATTTGGGATGAAGGAGTTAGCAGAG  
 Mazzaella\_membranacea: CATGCGTTGGAAAGAAAGATACCTATATTCAATGGAAGGTGTAACCGTG  
 Karenia\_brevis: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Jaxonville\_C3a: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Mexico\_Beach\_C5: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Piney\_Island\_A9: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Charlotte\_Harbor\_C2: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Charlotte\_Harbor\_A2: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Piney\_Island\_B4: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Mexico\_Beach\_B3: TATGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Appalachicola\_C6: TACGCGGTATCGTGAACGTTTCCTATACTCTATGGAAGGTGTCAATCATG  
 Karenia\_mikimotoi\_1: TATGCGTTACCGAGAACGTTTCTTTCTCAATGGAAGAGGTGAATCATG  
 Karenia\_mikimotoi: TATGCGTTATCGAGAACGTTTCTTTCTCAATGGAAGCGGTGAATCATG  
 Karlodinium: TATGAGGTATAGAGAAAGGTTTTTATTCTCCATGGAAGGGGTGAATCATG  
 Amphidinium: CGGTGATGCAACGCTCACCAGAGCGCACCCAGGGATAAAGGCTGGGCAGC  
 ST2SY33\_clone: CCAACGTTGGAGAGAAAGATTTGAGTTTGTTCGAGAAGCAGTTAAGCTTG  
 Prochlorococcus: CCAACGTTGGAGAGAAAGATTTGAGTTTGTTCGAGAAGCAGTTAAGCTTG  
 Thiobacillus: ACATCATGAACAAGTATGGTCGCCCATTTGCTGGGCTGCACCATCAAACCC  
 Synechococcus\_sp: CCAACGCTGGCGCGATCGCTTCCTGTTTGTTCGATGCCATTCAAAAG  
 ST2SY26\_clone: CNAGCGTTGGCAGAACCGCTTCGAATTCGTTGCGGAAGCCATCAAGCTGT  
 Synechococcus: ACCGGATGAACAAGTACGGCCGTCCTGCTGGGTTGCACCATCAAGCCG  
 Nitrobacter: ACAAGCTTAACAAGTACGGCCGCCCCGCTGTTGGGCTGCACCATCAAGCCG  
 Synechocystis: CATGCGTTGGCGCGATCGTTTCCTCTTCGTTCAAGAGCGATCGAAAAAG  
 Synechococcus\_PCC7002: CATGCGTTGGCGCGATCGCTTCCTGTTTCGTTCAAGAAGCTATCGAAAAAT  
 ST8SY15\_clone: CATGCGTTGGCGCGATCGCTTCCTATTCGTTCAAGAAGCCATTGAAAAGG  
 Trichodesmium: CATGCGTTGGCGCGATCGCTTCCTATTCGTTCAAGAAGCCATTGAAAAGG  
 ST5SY7\_clone: TATGAGATGGAGAGATAGATTCTTATTTGTTTCTGAAGCAATTTATAAAG  
 ST2SY2\_clone: TCAGCGTTGGCGGTGATCGTTTCCTATTCATTGCTGAGGCTACACACAAGT  
 ST6SY8\_clone: CATGCGTTGGCGGTGATCGTTTCCTTTTCTGTGCTGAGGCTATTTACAAGG  
 Volvox\_carteri: CGGTATTCAGGTTGAACGTGACAACTAAACA---AATATGGTCGTGGTC  
 Spinacia: TATGCGTTGGAGAGACCGTTTCCTATTTTGTGCCGAAGCTCTTTATAAAG  
 Asparagus: TATGCGTTGGCGAGACCGTTTCGTATTTTGTGCTGAAGCTCTTTATAAAG  
 Thalassia: TGGAAATCCAAGTGGAAAGAGATAGATTGAACA---AATACGGCCGCCCTC  
 Prymnesium: CATGCGTTACAGAGAGCGTTTCCTTTATTCAATGGAAGGTGTTAACCACG  
 Chrysochromulina\_sp: CATGCGTTACAGAGAGCGTTTCCTTTACTCAATGGAAGGTGTTAACCACG  
 Imantionia: CATGCGTTACAGAGAGCGTTTCCTTTACTCAATGGAAGGTGTTAACAACG  
 ST1CH3\_clone: TATGCGTTTCAGAGAGCGTTTCCTATACTCTATGGAAGGTGTTAACCACG  
 Helicosphaera: CATGCGTTACAGAGAGCGTTTCCTATACTCAATGGAAGGTGTTAACCACG  
  
 Emiliana\_huxleyi: CAGCTTGCTAACTGGTGAAGTTAAAGGTCACCTAACACAACCTGCG  
 ST8CH26\_clone: CTGCTTGTCAGACTGGTGAAGTTAAAGGTCACCTAACGCAACAGCT  
 Isochrysis: CTGCTGCAACAACCTGGTGAGGTTAAAGGTCACCTAACACAACCTGCA  
 Calcidiscus: CTGCTGCAACAACCTGGTGAGGTTAAAGGTCACCTAACGCAACAGCT  
 Cruciplacolithus: CTGCTGCAATGTCTGGTGAAGTTAAAGGTCACCTAATGCAACAGCT  
 Pleurochrysis: CAGCGGCAGTAAGTGGGGAAGTAAAAGGTCACCTAACGCAACCGCA  
 Pavlova: CTGCAGCTGGTTCTGGAGAGGTTAAAGGTCACCTAATGCAACAGCT  
 Exanthemachrysis: CAGCGGCAGCTTCTGGAGAAGTAAAGGTCACCTAACGCTACAGCT  
 Pyrenomonas: CTGCTGCTGGTACTGGTGAGATTAAGGTCACCTAACATTAAGCTGCA  
 Chromonas: CTGCTGCTGCTGCAGGTGAAGTTAAAGGACATTACTTTAACGTTACAGCT  
 Rhodella: CTTCTGCAAGTTCTGGTGAAGTTAAAGGTCATTACCTTAACGTCACAGCA  
 Dictyocha: CCGCAGCAGCTACTGGTGAAGTTAAAGGTTNNNTACCTTAATGCTACTGCT  
 ST6CH33\_clone: CAGCAGCTGCTTCTGGTGAAGTTAAAGGTTCTTACTTAAATGCTACTGCA  
 Heterosigma: AATCAGGTGTAATCCCTTACGCTAAAATGGGTTATTGGGATGCTAATTA-  
 Chattonella: CTTCTGCTGCTTACAGGTGAAGTTAAAGGTTCTTACTTAAACGTACAGCT  
 ST4CH31\_clone: CTTACAGTGCACCTGGTGAACAAAAGGTTTACCTAACATTAACAGCA  
 ST1CH4\_clone: CAGCAGCTAAAACCTGGTGCAGTTAAAGGTTCTTACTTAAACGTAACGGCT

## Appendix 2 (Continued)

ST3CH27\_clone: CTGCTACTGCAAGTGGTGAAGTTAAAGGTTCTTACTTAAACGTAACCTGCT  
Ectocarpus: CTGCAGCTGCAACAGGTGAAGTTAAAGGTTCTTATCTTAAACATTACAGCC  
Xanthonema: CTGCTGCTGCAACAGGTGAAGTTAAAGGTTCTTACTTAAACGTTACAGCT  
Vaucheria: CAGTAGCAGCTAGTGGTGAAGTTAAAGGTTTCATACCTTAATACAACCTGCA  
Heterococcus: TATTAGGTGCAACGGTAAAACCAAATTAGGCTTTTCAGGTAAAACTAC  
Aureoumbra: CCGTTTCAGTAACAGGTGAGGTTAAAGGTTCTTACTTAAACATTACTGCT  
Aureococcus: CCGCTGCTGCAACTGGTGAAGTTAAAGGTTCTTACTTAAACATTACTGCT  
Pelagococcus: CTGCTGCTGCAACTGGTGAAGTTAAAGGTTCTTACTTAAACATTACTGCT  
Cylindrotheca: CTTCTGCTGCTACAGGTGAAGTTAAAGGTTCTTATTTAAACGTTACAGCT  
Phaeodactylum: CTTCTGCTGCTACAGGTGAACTAAAGGTTTCATACCTAAACATTACAGCT  
ST4CH14\_clone: CTGCTGCTGCTACTGGTGAAGTTAAAGGTTCTTACTTAAACATTACTGCA  
ST6CH2\_clone: CTTTCAGCAGCTACTGGTGAAGTTAAAGGTTCTTACTTAAACGTTACTGCT  
Rhizosolenia: CTGCTGCCGCTACTGGTGAAGTTAAAGGTTCTTACTTAAACATTACTGCT  
Skeletonema: CATCAGCTGCAACTGGTGAAGTTAAAGGTTCTTACTTAAACATTACTGCT  
Thalassiosira: CAGCTGCTGCAACTGGTGAAGTTAAAGGTTCTTACTTAAACATTACTGCT  
Detonula: GTACTATTGGTCACCTGATGGTATTCAAGCTGGTGCTACAGCAAACCGT  
Chilomonas: CTTCTGCTTCTTCTGGTGAAGTTAAAGGTTCACTACTTAAACATTACTGCG  
Mazzaella\_membranacea: CTATTGCTGCTGCTGGTGAACCAAAGGGCATTATTTAAATTAACAGCA  
Karenia\_brevis: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Jaxonville\_C3a: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Mexico\_Beach\_C5: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Piney\_Island\_A9: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGACA  
Charlotte\_Harbor\_C2: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Charlotte\_Harbor\_A2: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Piney\_Island\_B4: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Mexico\_Beach\_B3: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Appalachicola\_C6: CTGCCGCAAAAACCGGTGAAATCAAAGGACATTATTTAAATGTCACGGCA  
Karenia\_mikimotoi\_1: CTGCTGCTAAGTCAGGGGAAATCAAAGGTCATTATTTAAACGTAACCTGCG  
Karenia\_mikimotoi: CTGCTGCTCAGTCAGGAGAAATAAAAGGTCATTACCTAAACATAACTGCG  
Karlodinium: CTTCTTGTTTAACGGGTGAGGTTAAAGGACATTATTTAAATGTTACTGGG  
Amphidinium: TTCTGTCGCTTCCCATGAGTATGGACGCTGCCGAACCTTTTGCCACCGCA  
ST2SY33\_clone: CTCAGCAAGAACTGGTGAAGTTAAAGGTCACCTAACCTGCTACCTGCC  
Prochlorococcus: CTCAGCGAGAACTGGAGAAGTTAAAGGTCACCTATCTAAATTTGACTGCT  
Thiobacillus: AAGCTGGGTTTGTGCGCCAAGAATATGGTCGCGCTTGCTATGAGGGTCT  
Synechococcus\_sp: CCCAGGCGGAAACCGGTGAAATCAAAGGTCACCTATTTGAACGTCACCGCT  
ST2SY26\_clone: CCGAGCAGGAGACCGGCGAGCGCAAGGGTCACCTACCTCAACGTCACCGCC  
Synechococcus: AAGCTCGGCTGAGCGGCAAGAATACGGCCGTGTTGCTATGAGTGCT  
Nitrobacter: AAGCTTGGTCTGTCCGGAAGAATACGGCCGCTGCTATGAAGCGCT  
Synechocystis: CCCAGGCTGAGACCAACGAAATGAAAGGTCACCTACCTGAACGTCACCGCT  
Synechococcus\_PCC7002: CCCAAGCTGAAACCAACGAAATGAAAGGTCACCTACCTAACGTCACCGCT  
ST8SY15\_clone: CCCAAGCAGAAACAGGTGAAATGAAAGGTCACCTACCTCAACGTAACCTGCC  
Trichodesmium: CCCAAGCAGAAACAGGTGAAATGAAAGGTCACCTACCTCAACGTAACCTGCC  
ST5SY7\_clone: CTCAAGCAGAAACAGGAGAAATGAAAGGTCATTACTTAAATGCTACTGCA  
ST2SY2\_clone: CTCAAGCAGAGACTGGTGAAGTTAAAGGTCACCTAACGTCACCTGCT  
ST6SY8\_clone: CACAAGGTGAAACTGGTGAAGTTAAAGGTCACCTAACGCAACTGCT  
Volvox\_carteri: TTTTAGGTTGTACAATCAAACCTAAATTAGGCTTTTCAGCTAAAACTAC  
Spinacia: CACAAGCCGAAACAGGCGAAATCAAAGGTCATTACTTGAATGCTACCGCG  
Asparagus: CACAAGCAGAAACAGGTGAAATCAAAGGCCATTACTTGAATGCAACTGCA  
Thalassia: TACTAGGATGACTATTTAAACCAAATTTGGGATTATCCGCGAAAACTAT  
Prymnesium: CAGCAGCAATGACTGGTGAAGTTAAAGGTCACCTAACGTAACGCGCA  
Chrysochromulina\_sp: CATCTGCTAATGTTGAGTTAAAGGTCACCTAACCTACTGCG  
Imantionia: CAGCTGCTCAGTGGTGAAGTTAAAGGTCACCTAACCTACTGCT  
ST1CH3\_clone: CAGCAGCAATGCTGTTGAGGTTAAAGGTCACCTAACCTACTGCT  
Helicosphaera: CAGCTGCAATGACAGGTGAAGTTAAAGGTCACCTAACCAACTGCT  
  
Emiliana\_huxleyi: GCAACAATGGAAGATATGTACGAACGTTACTTCCGAAGAGATCTAGG  
ST8CH26\_clone: TCAACTATGGAAGACATGTACGAGGTTCTGAGTCTGTTACGAATTAGG

## Appendix 2 (Continued)

Isochrysis: GCTACAATGGAAGATATGTACGAGCGTGCTGACTTCGCTAAGGAGTTAGG  
Calcidiscus: GCAACAATGGAAGATATGTATGAGCGTGCAGAATTCTGTAAAGAGCTTGG  
Cruciplacolithus: GCAACAATGGAAGATATGTATGAGCGCGCAGAATTCTCTAAGGAGCTTGG  
Pleurochrysis: GCGACGATGGAAGATATGTACGAGCGTGCTGAATTCGCTAAGGATTTAGG  
Pavlova: TCTACTATGGAAGAAATGTATACGCGTGCTGACTACGCTAAGGAGCTAGG  
Exanthemachrysis: GGTACTATGGAAGACATGTACGAGCGTGCTGACTACTCTAGAGATCTAGG  
Pyrenomonas: GGTACAATGGAAGATATGTACGAGCGTGCTGAATTCGTAAAGAAATCGG  
Chroomonas: GGTACAATGGAAGAAATGTATGCTCGTGCTGAATTCGTTCAGATTTAGG  
Rhodella: GCAACTATGGAAGATATGTACGAGCGTGCTGAATTCCTAAAGCAGTTGG  
Dictyocha: GGTACTATGGAAGNGTTTACGAACGTGCTGAATATGCTAAGGAACTTGG  
ST6CH33\_clone: GCGACTACGGAAGAAATGTACGTTCTGTCAGATTACGCTAAAGTCTGTTGG  
Heterosigma: --TTCTGTTAAAGATACAGATTTATTAGCTTTATTCCGCTTACCACACA  
Chattonella: GCTACAATGGAAGAAATGTACGAACGTGCAGAATATGCTAAGTCTGTTGG  
ST4CH31\_clone: GCTACAATGGAAGAAGTTTACAAACGTGGTGATTATGCGAAAGCAGTTGG  
ST1CH4\_clone: GCAACTATGGACGAAATGTATGAAAGAGCTGAATTCGCAAAAGAGTTGG  
ST3CH27\_clone: GGTACTATGGAGCAAATGTACGAACGTGCTGAATTCGCTAAATCATTGG  
Ectocarpus: GCAACTGTAGAGCAAATGTATGAACGTGCAGAGTATGCTCATGCAATTGG  
Xanthonema: GGTAACATGGAAGACATGTACGAACGTGCTGAATGCTAAGGAACTTGG  
Vaucheria: GGTACAATGGAAGAGATGTATGAACGTGCAGAATATGCTAAAAGTATAGG  
Heterococcus: GGTCTGTAGTATATGAAGGTCTTCTGGTGGTCTTGATTTCTAAAAGA  
Aureoumbra: GGTACTATGGAAGAAATGTACACACGTGCAGAGTACGCTAAGGAACTTGG  
Aureococcus: GCAACAATGGAAGAAATGATCATTCTGTCAGAGTACGCTAAAGAGTTAGG  
Pelagococcus: GGTAACAACGATGAAATGATCACTCGTGGTGAGTACGCTAAAGAGATCGG  
Cylindrotheca: GCTACTATGGAAGAAGTATACAAACGTCTGAGTATGCTAAAGAAGTAGG  
Phaeodactylum: GGTACTATGGAAGAAGTTTACAAACGTGCAGAATATGCTAAAACAGTAGG  
ST4CH14\_clone: GGTACAATGGAAGAAGTTTACAAACGTGCAGAATATGCTAAATCTGTAGG  
ST6CH2\_clone: GCCACTATGGAAGAAGTTTACAAACGTGCAGAATATGCTAAACAAGTTGG  
Rhizosolenia: GCAACATACGAAGAAGTAGAAAAACGTGCTGACTATGCTAAAGCTGTTGG  
Skeletonema: GCTACAATGGAAGAAGTATACAAACGTGCTGAGTATGCTAAAGCTGTTGG  
Thalassiosira: GCTACTATGGAAGAAGTATACAAACGTGCTGAGTATGCTAAAGCTATTGG  
Detonula: GTTGCTTTAGAAGCAATGGTTTTTCTCGTAACGAAGGTGCTGACTACTT  
Chilomonas: GGAACAATGGAAGACATGTATGAGAGGCTAATTTTGCTAAAAGAAATGG  
Mazzaella\_membranacea: GCTACAATGGAACAAATATATGAAAGAGCTGAATTTGCTAAAGAGCTTGG  
Karenia\_brevis: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Jaxonville\_C3a: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Mexico\_Beach\_C5: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Piney\_Island\_A9: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Charlotte\_Harbor\_C2: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Charlotte\_Harbor\_A2: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Piney\_Island\_B4: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Mexico\_Beach\_B3: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Appalachicola\_C6: GCGACGATGGAAGACATGTATGAAAGAGCGAATTTTCGCTACGGAAGCTAGG  
Karenia\_mikimotoi\_1: GCAACAATGGAGAACATGTATGAAAGAGCAAATTTTCGCTGGTGAAGCTTGG  
Karenia\_mikimotoi: GCAACAATGGAGGACATGTATGAAAGAGCAGATTTTCGCCGCCGAACTTGG  
Karlodinium: GCAACCATGGAAGATCTTTATGAACGTTTCAGACCTATGTTATCAACTAGG  
Amphidinium: GAGAAGGGTGCAGTCTTCTTCTGTCGTTCTTGATCTGTGGCCGTTGG  
ST2SY33\_clone: AACACTCCTGAAGAACTTTACGAAAGAGCTGAATTTGCAAAAGAGCTAGA  
Prochlorococcus: AACACTCCTGAAGAACTCTATGAAAGAGCTGAATTTGCAAAAGAGCTCGA  
Thiobacillus: GCGTGGTGGACTCGACTTCACTAAAGATGACGAAAACGTCAATAGCCAGC  
Synechococcus\_sp: CCCACCTGTGAAGAAATGTTGAAACGGGCAGAGTTTCGCTAAAGA---CTG  
ST2SY26\_clone: AACACTCCCGAAGAGATGTACGAGCGCGCTGAGTTCCGCCAAGGAAGCTCGG  
Synechococcus: GCGTGGCGGTCTGGACTTCACCAAGGACGACGAGAACATCAACTCCAGC  
Nitrobacter: ACGTGGCGGTCTGGACTTCACCAAGGACGACGAGAACATCAACAGCCAGC  
Synechocystis: GGCACCTGCGAAGAAATGATGAAACGGGCCGAGTTTGCCAAGGAAATTTGG  
Synechococcus\_PCC7002: GGCACCTGCGAAGAAATGCTCAAGCGGGCTGAATTCGCTAAGGAAATTCGG  
ST8SY15\_clone: CCTACCTGTGAAGAAATGTTAGAGCGTGACAGATTTTGCTAAGGAAATTTGG  
Trichodesmium: CCTACCTGTGAAGA---ATGTAGACGTGCAGTTTTTGCTAAGGAAATTTGG

## Appendix 2 (Continued)

ST5SY7\_clone: GGTACTTGTGAAGAGATGTTAAAAAGAGGTGAATGTGCTAAAGAATTAGG  
 ST2SY2\_clone: GGTCACGTGTGATGAGATGCTTAAGCGTGCAGCATTTGCTCGTGAGATCGG  
 ST6SY8\_clone: GGTACGAATGAGGAGATGATGAAGCGTGCTGAGTTCGCTGTTGATCTTGG  
 Volvox\_carteri: GGTCTGTCAGTTTACGAATGTTTACGTGGTGGTTTAGACTTTACTAAAGA  
   Spinacia: GGTACATGCGAAGATATGATGAAAAGGGCTGTATTTGCCAGAGAATTGGG  
   Asparagus: GGTACATGTGAAGAAATGATGAAAAGGGCCATATTTGCCAGAGAATTGGG  
   Thalassia: GGTAGAGCAGTTTATGAATGTCTACGTGGTGGACTGGATTTTACTAAAGA  
   Prymnesium: GCTACTATGGAAGATATGTACGAACGTGCAGAGTTCTGTAAAGAGCTAGG  
 Chrysochromulina\_sp: GCTACTATGGAAGATATGTACGAACGTGCTGATTTCCGCTAAAGAGCTAGG  
   Imantionia: GCTACGATGGAAGATATGTACGAGCGTGCAAACCTTCGCGAAAGATCTAGG  
   ST1CH3\_clone: TCTACAATGGAAGATATGTATGAGCGTGCTGATTTAGCATGTGAATTAGG  
   Helicosphaera: GCAACTATGGAAGATATGTACGAGCGTGCAGATTTTGCAAAAAGAACTTGG  
  
 Emiliana\_huxleyi: TTCAGTTATTGTAATGATTGACCTTGTAAATTG---GTTATA-----C  
 ST8CH26\_clone: TTCAGTTATTGTAATGATTGACCTTGTAA---TCGGTTATA-----C  
   Isochrysis: TTCAGTTATTGTTATGATCGACCTTGTAAATCG---GTTATA-----C  
   Calcidiscus: TTCTGTAATTGTGATGATCGACCTTGTAAATTG---GTTATA-----C  
 Cruciplacolithus: TTCAGTAATTACAATGATTGACCTTGTAAATTG---GTTACA-----C  
   Pleurochrysis: TTCAGTTATTGTTATGATTGACCTTGTGATTG---GTTATA-----C  
   Pavlova: TTCAATCATTGTTATGATTGACCTTGTATTGGA---TACA-----C  
 Exanthemachrysis: TTCAATCATTGTTATGATCGACCTTGTAAATCG---GATACA-----C  
   Pyrenomonas: TACTGTATTTGTATGATCGACCTTGTAAATCGGT---TACA-----C  
   Chroomonas: CTCTGTAATTTGTATGATCGACCTTGTAAATGGT---TATA-----C  
   Rhodella: AAGCATCATTGTTATGATCGACCTTGTAAATGGT---TATA-----C  
   Dictyocha: TACTATTGGTATTATGAATGACCTTGGTATGG---GTTATA-----C  
 ST6CH33\_clone: TACTATCATTATCATGATTGACCTTGTAA---TTGGTTACA-----C  
   Heterosigma: ACCTGGAGTAGACCTGTGGAAGCTGCAGCGGCAGTAGC--AGGTGAATC  
   Chattonella: TTCTGTAATTATCATGATTGACCTTGTAAATTG---GTTACA-----C  
 ST4CH31\_clone: ATCTGTAATTGTATGATGATTGATTAGTAA---TGGGTTACA-----C  
   ST1CH4\_clone: ATCAGTTATTATCATGATTGACTTAGTAA---TTGGTTACA-----C  
 ST3CH27\_clone: ATCTGTAATTATTATGATCGATTTAGTAA---TTGGTTATA-----C  
   Ectocarpus: TTCAGTTATTGTTATGATCGATTTAGTTATTGGTTATAC--AGCAATTCA  
   Xanthonema: TTCAATCATTGTTATGATCGACCTTGTAAATTG---GTTACA-----C  
   Vaucheria: CTCAATTATTGTAATGATTGACCTTGTAAATGGTTATAC--TGCTATTCA  
   Heterococcus: TGATGAAAACATTAACCTCACAACTTTCATGCGTTGGAGAG-----A  
   Aureoumbra: TTCAATTATTGTAATGATCGATTTAGTTATCG---GTTACA-----C  
   Aureococcus: TTCTATCATTATCATGATTGACTTAGTTATTG---GTTACA-----C  
   Pelagococcus: TTCTGTAATCGTAATGATTGACTTAGTAAATTG---GTTACA-----C  
   Cylindrotheca: TTCTATCATTATCATGATCGATTTAGTTATGG---GTTACA-----C  
   Phaeodactylum: TTCTATCGTTGTTATGATCGATTTAGTTATGG---GTTATA-----C  
 ST4CH14\_clone: TTCTATCATTGTAATGATCGATTTAGTTA---TGGGTTACA-----C  
   ST6CH2\_clone: TTCTGTAATTATCATGATCGATTTAGTAA---TGGGTTATA-----C  
   Rhizosolenia: TTCTGTAGTTGTAATGATCGATTTAGTAATGG---GTTACA-----C  
   Skeletonema: TTCTATCGTTGTTATGATCGATTTAGTAATGG---GTTACA-----C  
 Thalassiosira: TTCTGTCTTGTAATGATCGATTTAGTAATGGTTACAC--TGCAATTCA  
   Detonula: TAATAACCAAGTAGGTCCTCAAATCTTACGTGACGCAGCTAAGACATGTG  
   Chilomonas: TAGTGTATCTGTATGATCGATCTTGTATTGGA---TATA-----C  
 Mazzaella\_membranacea: TAGTATCATTGTAATGATTGACCTTGTAAATTG---GTTATA-----C  
   Karenia\_brevis: TTCCATAATTGTAATGATAGATCTTGTAA---ATA-----C  
   Jaxonville\_C3a: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C  
   Mexico\_Beach\_C5: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C  
   Piney\_Island\_A9: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C  
   Charlotte\_Harbor\_C2: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C  
   Charlotte\_Harbor\_A2: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C  
   Piney\_Island\_B4: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C  
   Mexico\_Beach\_B3: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C  
   Appalachicola\_C6: TTCCATAATTGTAATGATAGATCTTGTAA---TTGGTTATA-----C

## Appendix 2 (Continued)

Karenia\_mikimotoi\_1: TTCAATCATTGTGATGATCGATCTTGTA---TTGGGTATA-----C  
 Karenia\_mikimotoi: TTCGGTCATTGTGATGATCGACCTTGTAATTGGGT---ATA-----C  
 Karlodinium: GTCGGTTATTTAATGATTGATCTTGTTATTGGTT---ATA-----C  
 Amphidinium: TCTTGTGGGTGCTGGTTTGGCGCTCACGGAACCTCGCGCTTGG-----C  
 ST2SY33\_clone: TATGCCAATCATCATGCATGATTATATAACTGGCGGTTTTA-----C  
 Prochlorococcus: TATGCCAATCATCATGCATGATTACATAACTGGTGGTTTTA-----C  
 Thiobacillus: CTTTCATGCGCTGGAGGCAGCGTTTCGACTTCGTCATGG--AGGCCATCC  
 Synechococcus\_sp: GAATGCCATCATCATGCACGACTTCTCACCGCTGGCTTCA-----C  
 ST2SY26\_clone: CATGCCGATCATCATGCACGACTTCATCACCGGTGGCTTCA-----C  
 Synechococcus: CCTTCCAGCGTTGGCAGAACCCTTCGAATTCGTTGCGG--AAGCCATCA  
 Nitrobacter: CCTTCATGCGCTGGCGCGATCGTTTCGATTTTCGTGATGG--AGGCTGTTC  
 Synechocystis: CACCCCATCATCATGCATGACTTCTTCACCGCGGTTTTA-----C  
 Synechococcus\_PCC7002: CACTCCCATCATCATGCACGACTTCTTAACCTGGTGGTTTTA-----C  
 ST8SY15\_clone: CACTCCAATCATTATGCACGACTACCTAACTGGTGGTTTTA-----C  
 Trichodesmium: CACTCCAATCATTATGCACGACTATCTCACAGGTGGTTTTA-----C  
 ST5SY7\_clone: TGTTCCTATTATTATGCATGACTATTTAGCAGGTGGATTTA-----C  
 ST2SY2\_clone: TGCACCCATTATTATGCATGACTACCTAACAGGTGGTTTTA-----C  
 ST6SY8\_clone: TATGCCATTATTATGCACGACTACCTTACTGGTGGTTTTA-----C  
 Volvox\_carteri: CGATGAAAACGTTAACTCACAACCATTTCATGCGTTGGAGAG-----A  
 Spinacia: CGTTCCTATTGTAATGCATGACTACTTAACAGGGGGATTCA-----C  
 Asparagus: AGTTCATCGTAATGCATGACTACTTAACAGGGGGATTCA-----C  
 Thalassia: TGATGAAAACGTAAATTTCCAGCCATTTATGCGTTGGAGAG-----A  
 Prymnesium: TTCTGTTATCGTAATGATTGACTTAGTAATCG---GTTACA-----C  
 Chrysochromulina\_sp: TTCGATTATTGTAATGATTGACCTTGTAATTG---GTTATA-----C  
 Imantionia: TTCAATCATTGTAATGATTGACCTTGTAATCG---GTTACA-----C  
 ST1CH3\_clone: TTCAATCATTGTAATGATTGACCTTGTA---TCGGTTATA-----C  
 Helicosphaera: TTCAGTTATTGTAATGATTGACCTTGTAATTG---GTTACA-----C  
  
 Emiliana\_huxleyi: AGCTATCCAATCAATGGGTAAGTGGTCACGTGATAACGATGTGATCCTTC  
 ST8CH26\_clone: AGCAATTCAAACAATGGGTAAGTGGTGTGTAAGAGAGATATGATCCTTC  
 Isochrysis: TGCTATCCAGACAATGGGTCGTTGGGCTCGTAAGTCTGATGTTATCCTTC  
 Calcidiscus: AGCTATCCAATCAATGGCTAAATGGTCACGTAAGTATGATATGATCCTTC  
 Cruciplacolithus: AGCTATCCAATCAATGGCTAATGGGCACGTAAGTCTGATATGATCCTTC  
 Pleurochrysis: AGCAATCCAATCGATGGCTATCTGGGCACGTAAGACAGATATGATCCTTC  
 Pavlova: TGCTATCCAACAATGGGTATCTGGGCTCGTAAGAATGATACAATTCTTC  
 Exanthemachrysis: AGCTATCCAAGTATGGCTAAGTGGGCTCGTAAGTACGATATGATCCTTC  
 Pyrenomonas: AGCTATCCAAGTATGGGTATCTGGGCTCGTAAGAACAGTATGATCCTTC  
 Chromonas: AGCTATCCAATCTATGGGTATTTGGGCTCGTAAGAACAGTATGATCCTTC  
 Rhodella: TGCAATTCAAAGTATGGCTATTTGGGCTCGTAAAAACGATATGATCCTTC  
 Dictyocha: AGCAATTCAATCAATGCGTATTTGGGCTCGTAAGAATGATATGATCCTTC  
 ST6CH33\_clone: AGCTATCCAATCTATGGCTGTTTGGGCTCGTGAAAACGATATGATTTTAC  
 Heterosigma: TTCTACCGCTACTTGGACTGTAGTATGGACAGATTTATTAACCTGCAT---  
 Chattonella: AGCTATCCAAGTATGGCTATCTGGTCTCGTAAAAACGACATGATTTTAC  
 ST4CH31\_clone: AGCAATTCAAAGTATTTGACTTTGGGCTCGTGAGAATGATATGCTTTTAC  
 ST1CH4\_clone: TGCTATCCAACCTATGGCATTCTGGGCTCGTAAGAATGATATGATCCTTC  
 ST3CH27\_clone: TGCAATTCAATCAATGGCTAATTTGGGCTCGTAAACGATATGATCCTTC  
 Ectocarpus: AAGTATGGCAATCTGGGCACGAAAAGCTGAAGTATTTTACATTTAC---  
 Xanthonema: AGCGATCCAATCAATGGGTATTTGGGCTCGTAAAAATGACATGATCCTTC  
 Vaucheria: ATCTATGTCAATTTGGGCTAGAAAAGCTGATATGATTTTACATTTAC---  
 Heterococcus: ACGTTTTCTTTACTGTATGGAAGGTGTAACCCTGCGGCTGCAGCAACTG  
 Aureoumbra: AGCTATCCAAGTATGGCTATTTGGGCTCGTAATAACGATATGCTTTTAC  
 Aureococcus: AGCTATCCAACCTATGGCTATCTGGTCTCGTGAGAACGATATGCTTTTAC  
 Pelagococcus: TGCTATCCAACCTGCTGCAATCTGGGCTCGTTCAAACGACATGATTTTAC  
 Cylindrotheca: AGCAATTCAAAGTATGGCTTTATGGGCTCGTGAAAATGATATGCTTTTAC  
 Phaeodactylum: TGCAATCCAATCAGCTGCGATTTGGGCGCGTGATAATGATTTAATTTTAC  
 ST4CH14\_clone: AGCTATCCAATCAATGCAATCTGGGCACGTAAGAACGATATGCTTTTAC  
 ST6CH2\_clone: TGCGATTCAAAGTATTCATTATGGTCTCGTGAAAACGATATGCTTTTAC

## Appendix 2 (Continued)

Rhizosolenia: AGCGATTCAAAGTATTGCTCACTGGGCTCGTGACAACGATATGGTATTAC  
 Skeletonema: TGCAATTCAATCAATTGCATACTGGGCTCGTGAAAACGATATGTTATTAC  
 Thalassiosira: ATCAATTGCATACTGGGCTCGTGAAAACGATATGTTATTACATTTAC---  
 Detonula: GTCCTTTACAAACAGCTTTAGATCTATGGAAAGATATTAGTTTCAACTAT  
 Chilonomas: TGCCATTCAGTCTATGGCTATTTGGGCTCGTAAAAATAGTATGATATTAC  
 Mazzaella\_membranacea: AGCTATTCAATCAATGGCTGTTTGGGCTCGTAAAAATGATATGATTCTTC  
 Karenia\_brevis: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Jaxonville\_C3a: GGCGATAACAATCAATGGCTTATTGGGCTCGCAATAATGATACTATGCTTC  
 Mexico\_Beach\_C5: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Piney\_Island\_A9: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Charlotte\_Harbor\_C2: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Charlotte\_Harbor\_A2: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Piney\_Island\_B4: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Mexico\_Beach\_B3: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Appalachicola\_C6: AGCGATAACAATCAATGGCTTACTGGGCTCGCAATAATGATACTATGCTTC  
 Karenia\_mikimotoi\_1: TGCAATTCAATCAATGGCGTACTGGGCGGACGAAATGACGTTATTCTTC  
 Karenia\_mikimotoi: TGCGATTCAATCAATGGCCTACTGGGCGGTCGAAATGACATTCTTCTAC  
 Karlovinium: TGCAATTCAATCAATTACACAATGGTCGAGAAAACATGATATGCTATTAC  
 Amphidinium: AAAGAAGTCAGGCAAGCGCAGTATTGTTGCACGCAAGGCATTTGGACCAGT  
 ST2SY33\_clone: TGCAAACTACTGGATTAGCAAACGGTGTGCGTAAAAATGGCATGCTTCTAC  
 Prochlorococcus: TGCAAACTACTGGATTAGCTAATTGGTGTGCGTAAAAATGGCATGCTTCTGC  
 Thiobacillus: AGAAGGCCGAGGCCGAGACCGGAGAGCGTAAGGGGCACTACCTGAACGTT  
 Synechococcus\_sp: GGCCAACACCACCTCTCGAAAGGGTGCCGTGACAACGGCATGTTGCTAC  
 ST2SY26\_clone: GGCCAACACCAGTTTGTGCAAGTGGTGCCGCAAGAACGGCATGTTGCTGC  
 Synechococcus: AGCTGTCCGAGCAGGAGACCGGTGAGCGCAAGGGTCACTACCTCAACGTC  
 Nitrobacter: AAAAAGCCGAACACGAGACCGGCGAGCGAAAGGTCACTATCTCAACGTC  
 Synechocystis: TGCCAACACCACCTCGCTCGTGGTGTGCGGACAACGGCATTTTGTCTCC  
 Synechococcus\_PCC7002: TGCGAATACTACCTTTCGCAAGTGGTGTGCGTATAACGGCGTCTGCTCC  
 ST8SY15\_clone: AGCTAATACAACCTTTAGCGAAATGGTGCCGTGTAATGGTGTATTATTAC  
 Trichodesmium: AGCTAATACAACCTTTAGCGAAATGGTGCCGTGTAATGGTGTATTATTAC  
 ST5SY7\_clone: TGCAAACTACTACTAGCTAAACTGTAGAGATACTGGATTATTATTAC  
 ST2SY2\_clone: TGCTAACACTACGCTAGCAATGTACTCTCGTGATAACGGTCTACTTCTAC  
 ST6SY8\_clone: ATCTAACACTACTCTTTCTAACTACTGTGCGTACAACGGTCTTCTCTCTCC  
 Volvox\_carteri: CCGTTTCCTTTTTCGTAGCTGAAGCTATTTACAAAGCACAAGCAGAAACAG  
 Spinacia: TGCAAACTACTACCTTGTCTCATTATTGCCGAGATAATGGTCTACTTCTTC  
 Asparagus: TGCAAACTACTACTTTGGCTCATTATTGCCGCGACAATGGTCTACTTCTTC  
 Thalassia: CCGTTTCCTATTTTGTGCCGAAGCCATTTTAAAGCGCAAGACGAAACAG  
 Pymnesium: AGCTATCCAGTCAATGGCTAAATGGTCACGTAAAACCTGATGATCCTTC  
 Chrysochromulina\_sp: TGCTATTCAATCAATGGCTAAGTGGTGTGCGTAAGACTGATGTAATCCTTC  
 Imantionia: TGCTATTCAAGTCAATGCTCACTGGGCGGTCGATAACGATGTGATCCTTC  
 ST1CH3\_clone: TGCTATCCAATCAATGGCTAAGTGGTCTCGTAAGAAAGATATGCTTCTTC  
 Helicosphaera: GGCTATCCAATCAATGGGTAAATGGTCTCGTAAGTACGATGTGCTTCTTC  
  
 Emiliana\_huxleyi: ACCTTCACCGTGCGGGTAACTCAACTTACTCACGTGAGAAGAACCACGGT  
 ST8CH26\_clone: ACTTACACCGTGCTGGTAACTCAACTTACTCACGTGAGAAGAACCACGGT  
 Isochrysis: ACCTTCACCGTGCAGGTAACCTCAACTTACTCTCGTCAAAAGAGCCACGGT  
 Calcidiscus: ACCTTCACCGTGCTGGTAACTCAACTTATTCTCGTCAAAAGATCATGGT  
 Cruciplacolithus: ACCTTCACCGTGCAGGTAACCTCAACTTACTCGGTCAAAAGAGTACGGT  
 Pleurochrysis: ACCTTCACCGTGCGGGTAACTCAACTTACTCACGTCAAAAGACTCATGGT  
 Pavlova: ACCTTCACCGTGCTGGTAACTTACTTACTCTCGTCAAAAGAACCACGGT  
 Exanthemachrysis: ACCTTCACCGTGCTGGTAACTCAACTTACTCTCGTCAAAAGAACCACGGT  
 Pyrenomonas: ACTTACACCGTGCTGGTAACTTACTTACTCTCGTCAAAAGACTCACGGT  
 Chromonas: ACCTACACCGTGCAGGTAACCTTACATATTCCCGTCAAAAGACACATGGT  
 Rhodella: ACTTACACCGTGCAGGAACTTACTTATTCTCGTCAAAAGATCATGGT  
 Dictyocha: ACTTACACCGTGCAGGTAACCTTACATATGCCGTCAAAAGAACCATGGA  
 ST6CH33\_clone: ATTTACACCGTGCTGGTAACTTACTTATGCTCGTCAAAAGATCACGGT  
 Heterosigma: -----GTGATGTTTATCGTGCAAAAGCTTACCGTGTGGACCCTGTA

## Appendix 2 (Continued)

Chattonella: ACTTACACCGTGCAGGTAACCTACTTACGCTCGTCAAAAGAACCACGGT  
ST4CH31\_clone: ATTTACACCGTGCAGGTAACCTACTACATATGCTCGTCAAAAAAATCATGGC  
ST1CH4\_clone: ACTTACACCGTGCAGGTAACCTACTTACGCTAGACAAAAAGAACATGGT  
ST3CH27\_clone: ACTTACACCGTGCAGGTAACCTACTTATGCACGTCAAAAAAACCATGGT  
Ectocarpus: -----ATCGTGCAGGTAACCTACTTATGCTCGTCAAAAAAACCATGGT  
Xanthonema: ACTTACACCGTGCCGGTAACCTCACTTACGCACGTCAAAAAAACCACGGT  
Vaucheria: -----ACCGTGCAGGTAATTCTACATATGCACGTCAAAAAAATCATGGG  
Heterococcus: GTGAAGTAAAAGGTTCTTACCTAACGCTACTGCTGGAAACATGGAAGAA  
Aureoumbra: ACTTACACCGTGCAGGTAACCTCAACATATGCACGTCAAAAGACTCATGGT  
Aureococcus: ATTTACACCGTGCAGGTAACCTCACTTACGCTCGTCAAAAGAGTCACGGT  
Pelagococcus: ATTTACACCGTGCAGGTAACCTCACTTACGCTCGTCAAAAAAACCACGGT  
Cylindrotheca: ATTTACACCGTGCAGGTAATTCTACTTATGCTCGTCAAAAAAATCATGGG  
Phaeodactylum: ATTTACACCGTGCAGGTAACCTCACTTACGCGCGTCAAAAAAATCATGGT  
ST4CH14\_clone: ATTTACACCGTGCAGGTAACCTACTACATACGCTCGTCAAAAGAACCACGGT  
ST6CH2\_clone: ATTTACACCGTGCAGGTAACCTACTACATATGCACGTCAAAAGAACCCACGGT  
Rhizosolenia: ATTTACACCGTGCAGGTAACCTACTTATGCTCGTCAAAAGAATCATGGT  
Skeletonema: ACTTACACCGTGCAGGTAACCTACTACATACGCTCGTCAAAAGAATCATGGT  
Thalassiosira: -----ACCGTGCAGGTAACCTACTTACGCTCGTCAAAAGAATCATGGT  
Detonula: ACTTCTACGATACAGCTGATTTTCGCTGAAACAGCTACTGCAAAACAGATA  
Chilomonas: ATTTACACCGTGCAGGTAACCTCCACATACTCTAGGCAAAAGATTCATGGA  
Mazzaella\_membranacea: ATTTGCATCGTGCAGGTAACCTACTTATTCTCGTCAAAAAAATCATGGT  
Karenia\_brevis: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Jaxonville\_C3a: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Mexico\_Beach\_C5: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Piney\_Island\_A9: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Charlotte\_Harbor\_C2: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Charlotte\_Harbor\_A2: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Piney\_Island\_B4: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Mexico\_Beach\_B3: ACTTGCATCGTGCCAGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Appalachicola\_C6: ACTTGCATCGTGCCGGTAATTCTACGTATTCTCGACAAAAAATCATGGT  
Karenia\_mikimotoi\_1: ATTTACACCGTGCAGGAACTCCACGTACTCTCGCCAGAAAAATCACGGA  
Karenia\_mikimotoi: ATTTACATCGTGCCGGAACTCCACGTACTCTCGCCAGAAAAATCACGGA  
Karlodinium: ATTTGCATCGTGCAGGTAATTTCGCGGTATGCAAGGCAAAAAGATCATGGT  
Amphidinium: CCAGCAGATACGCAGACCTGTCCCTCACCGAAGAGCAGCTCATCCAACAG  
ST2SY33\_clone: ATATTTCATAGAGCGATGCATGCTGTTATTGATAGACATCCAAAACACGGT  
Prochlorococcus: ATATTTCACAGAGCTATGCATGCTGTTATTGATAGACATCCAAAGCATGGT  
Thiobacillus: ACCGCCCCGACTCCGGAGGAAATGTACAAGCGTGCCGAGTACGCCAAGGA  
Synechococcus\_sp: ACATTACCGTGCATGACGCGCGTGATGGACCGTCAGAAAAACCACGGC  
ST2SY26\_clone: ACATCCACCGCGCCATGCACGCGGTGATCGACCGTCACCCCAAGCACGGC  
Synechococcus: ACCGCCAACACTCCGAAGAGATGTATGAGCGCGCTGAGTTCGCCAAGGA  
Nitrobacter: ACCGCGCAACGCCTGAGGAAATGTACAAGCGCGCCGAGTATGCCAAGGA  
Synechocystis: ATATTACCGGGCAATGCACGCGGTAGTTGACCGTCAGAAAAACCACGGG  
Synechococcus\_PCC7002: ACATCCACCGGGCAATGCACGCGGTAATCGACCGTCAGAAGAACCACGGT  
ST8SY15\_clone: ACATCCACCGGGCAATGCACGCGGTATTGACCGTCAGAAAGCCCACGGT  
Trichodesmium: ACATCCACCGGGCAATGCACGCGGTATTGACCGTCAGAAAGCCCACGGT  
ST5SY7\_clone: ATATTTCATCGTGCATGCATGCGGTTATTGATAGACAAAAAATCATGGT  
ST2SY2\_clone: ACATTACCGTGCATGCACGCGGTAATTGACCGTCAGCGTAACCACGGT  
ST6SY8\_clone: ACATTACCGTGCATGCACGCGGTAATTGACCGTCAGCGTAACCACGGT  
Volvox\_carteri: GTGAAGTAAAAGGTCATTATTTAACGCTACAGCTGGTACATGCGGAAGAA  
Spinacia: ACATCCACCGTGCATGCACGCGGTTATTGATAGGCAGAAGAATCATGGT  
Asparagus: ACATCCACCGCGCAATGCATGCTGTTATTGATAGACAGAAAAATCATGGT  
Thalassia: GTGAAATCAAAGGACATTACTTGAATGTTACTGACGAGTACGTTGAAAGAA  
Prymnesium: ACTTACACCGTGCAGGTAACCTCACTTACTCACGTCAAAAAAACCACGGT  
Chrysochromulina\_sp: ACTTACACCGTGCAGGTAACCTCACTTACTCACGTCAAGAACCACGGT  
Imantionia: ACTTACACCGTGCAGGTAACCTCACTTATTACGTCAGAAAAACCACGGT  
ST1CH3\_clone: ACTTACACCGTGCAGGTAACCTCACTTACTCACGTCAAGAACCACGGT  
Helicosphaera: ACCTACACCGTGCAGGTAACCTACTTACTCTCGTCAAAAGTCACATGGT



## Appendix 2 (Continued)

Emiliana\_huxleyi: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGTCAGGTTGTGACCA  
ST8CH26\_clone: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGTCAGGTTGTGATCA  
Isochrysis: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGTCAGGTTGTGACCA  
Calcidiscus: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGTCAGGTTGTGACCA  
Cruciplacolithus: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGTCAGGTTGTGATCA  
Pleurochrysis: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGTCAGGTTGTGACCA  
Pavlova: ATGAACTTCCGTGTTATCTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Exanthemachrysis: ATGAACTTCCGTGTAATTTGTAAGTGGATGCGTATGTCAGGTTGTGACCA  
Pyrenomonas: ATGAACTTCCGTGTTATCTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Chromonas: ATGAACTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Rhodella: ATGAACTTCCGCGTAATCTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Dictyocha: ATTAACTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGATCA  
ST6CH33\_clone: ATTAACTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Heterosigma: CCAAGTGCAGCTGACCAGTACTTCCGATACATCGCATACGAGTGTGACCT  
Chattonella: ATTAACTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
ST4CH31\_clone: ATGAATTTCCGTGTAATTTGTAAGTGGATGCGTATGTCAGGAGTTGACCA  
ST1CH4\_clone: ATTAACTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
ST3CH27\_clone: ATTAACTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Ectocarpus: ATTAATTTCCAGAGTTATCTGTAAGTGGATGCGTATGTCAGGTTGTGACCA  
Xanthonema: ATTAACTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Vaucheria: ATCAATTTCCGTGTAATTTGTAAGTGGATGCGTATGGCAGGTTGATGATCA  
Heterococcus: ATGTACACACGTGCTGAATACGCAAAAAGAAATGGTTCAATCATTGTGAT  
Aureoumbra: ATTAATTTCCGTGTAATTTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Aureococcus: ATTAACTTCCGTGTAATCTGTAAGTGGATGCGTATGGCTGGTGTGACCA  
Pelagococcus: ATCAACTTCCGTGTAATCTGTAAGTGGATGCGTATGGCTGGTGTGATCA  
Cylindrotheca: ATTAACTTCCGTGTAATTTGTAAGTGGATGCGTATGTCAGGTTGATGATCA  
Phaeodactylum: ATCAACTTCCGTGTTATTTGTAAGTGGATGCGTATGTCAGGTTGATGATCA  
ST4CH14\_clone: ATCAACTTCCGTGTTATCTGTAAGTGGATGCGTATGTCAGGTTGATGATCA  
ST6CH2\_clone: ATTAACTTCCGTGTTATCTGTAAGTGGATGCGTATGTCAGGTTGATGATCA  
Rhizosolenia: ATTAACTTCCGTGTTATCTGTAAGTGGATGCGTATGTCAGGTTGATGATCA  
Skeletonema: ATTAACTTCCGTGTTATCTGTAAGTGGATGCGTATGTCAGGTTGATGATCA  
Thalassiosira: ATTAACTTCCGTGTTTTCTGTAAGTGGATGCGTATGTCAGGTTGATGATCA  
Detonula: ATTAATTTACTTTTTATATATATTTAAGGAGTATTTGAATAGTGAGACTTAC  
Chilomonas: ATGAACTTCCGTGTCATTTGTAAGTGGATGCGTATGGCTGGTGTGATCA  
Mazzaella\_membranacea: ATGAATTTCCGAGTAATATGTAAGTGGATGCGTATGGCAGGTTGTGATCA  
Karenia\_brevis: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Jaxsonville\_C3a: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Mexico\_Beach\_C5: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Piney\_Island\_A9: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Charlotte\_Harbor\_C2: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Charlotte\_Harbor\_A2: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Piney\_Island\_B4: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Mexico\_Beach\_B3: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Appalachicola\_C6: ATGAATTTCCGCGTCATCTGTAAGTGGATGCGAATGGCTGGTGTGATCA  
Karenia\_mikimotoi\_1: ATGAATTTCCGTGTAATTTGTAAGTGGATGCGTATGGCAGGTTGTGATCA  
Karenia\_mikimotoi: ATGAATTTCCGTGTAATTTGTAAGTGGATGCGTATGGCAGGTTGTGATCA  
Karlodinium: ATCAATTTCCGTGTAATATGTAAGTGGATGAGAAATGGCGGGCTGAGACCA  
Amphidinium: GGTACAGCATGTGCTTGTGGCATAATCATGAAGCAAAAGGCTGGCTACGA  
ST2SY33\_clone: ATCCACTTCCAGGTTCTAGCAAAATGTTTGGAGACTCTCCGGAGGAGATCA  
Prochlorococcus: ATTCACTTCCAGGTTCTAGCAAAATGTTTGGAGACTATCTGGAGGAGACCA  
Thiobacillus: AATCGGCGCACCCATCATGCACGATTACATCACCGGCTTCTGCGG  
Synechococcus\_sp: ATCCACTTCCGTGCTTGGCCAAATGTTTGGCGGATGTCAGGCGGTGACCA  
ST2SY26\_clone: ATTCACTTCCGCGTTCTCGCCAAGTGTCTGCGTCTGTCCGGTGGTGTGACCA  
Synechococcus: ACTCGGCATGCCGATCATCATGCACGACTTACATCACCGGTTGGCTTCCAGG  
Nitrobacter: AATCCGCGCTCCGATCATCATGCACGATTACCTCGCTGGCGGGCTTTCGG  
Synechocystis: ATCCACTTCCGGTTTTGGCCAAGTGTCTGCGTCTGTCCGGCGGTGACCA

## Appendix 2 (Continued)

Synechococcus\_PCC7002: ATTCACTTCCGGTTCCTCGCTAAGTGTCTCCGCCTCTCTGGTGGTGACCA  
ST8SY15\_clone: ATTCACTTCCGGGTTTTAGCTAAGTGCTTGAGAATGTCTGGTGGTGACCA  
Trichodesmium: ATTCACTTCCGGGTTTTAGCTAAGTGCTTGAGAATGTCTGGTGGTGACCA  
ST5SY7\_clone: ATTCACTTCCGTGTTTTAGCTAAGGCTTTACGTTTATCTGGTGGTGATCA  
ST2SY2\_clone: ATTCACTTCCGTGTAATCTGTAAGGCTTACGTATGTAGGCTGGTGACCA  
ST6SY8\_clone: ATTCACTTCCGTGTTCTCGCAAGGCTCTCCGTCTTTCTGGTGGTGACCA  
Volvox\_carteri: ATGTTAAAACGTGCTCAATGTGCTAAAGAAGTGGTGTACCAATTATCAT  
Spinacia: ATGCATTTCCGTGTACTAGCTAAAGCATTACGTATGTCTGGTGGAGATCA  
Asparagus: ATGCATTTCCGTGTACTAGCTAAAGCATTACGTATGTCTGGTGGAGATCA  
Thalassia: ATGATCAAAAGAGCTGTATGTGCCAGAGAATTGGGAGTTCCATCGTAAT  
Prymnesium: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGGCTGGTGGTGACCA  
Chrysochromulina\_sp: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGGCTGGTGGTGACCA  
Imantionia: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGGCTGGTGGTGACCA  
ST1CH3\_clone: ATGAACTTCCGTGTAATCTGTAAGTGGATGCGTATGGCTGGTGGTGACCA  
Helicosphaera: ATGAACTTCCGAGTAATTTGTAAGTGGATGCGTATGGCTGGATGTGACCA  
  
Emiliana\_huxleyi: CATTACGCAGGTACTGTAGTAGGTAAGCTAGAAGGTGATCCACTAATGA  
ST8CH26\_clone: CATTACGCCTGGTACAGTAGTTGGTAAGCTAGAAGGTGATCCATTAATGA  
Isochrysis: CATTACGCAGGTACAGTAGTTGGTAAGCTAGAAGGTGATCCCTTAATGA  
Calcidiscus: TATCCACGCTGGTACAGTAGTAGGTAAGCTAGAAGGTGATCCCTAATGA  
Crucioplacolithus: CATTATGCAGGTACGGTAGTAGGTAAGTTAGAAGGTGATCCCTTAATGA  
Pleurochrysis: CATCCACGCGGGTACAGTAGTAGGTAAGTTAGAAGGTGACCCCTAATGA  
Pavlova: CATTACGCAGGTACCGTAGTAGGTAAGCTTGAAGGGGATCCACTAATGG  
Exanthemachrysis: CATTACGCCTGGTACTGTTGTAGGTAAGCTTGAAGGTGATCCCTAATGG  
Pyrenomonas: TATTCACGCTGGTACAGTTGTAGGTAAGTTAGAAGGGAGATCCCTAATGG  
Chroomonas: TATCCACGCTGGTACAGTAGTTGGTAAGCTTGAAGGAGATCCCTTAATGG  
Rhodella: TATTCACGCTGGTACAGTAGTAGGAAAATTAGAAGGGGATCCCTTAATGA  
Dictyocha: TATCCATGCGGGTACAGTTGTTGGTAAATTAGAAGGTGATCCCTTAATGG  
ST6CH33\_clone: TATCCACGCTGGTACTGTAGTAGGTAAGTTAGAAGGTGATCCATTAATGG  
Heterosigma: TTTTGAAGAAGGTTCTTTAGCTAACATGACTGCATCTATCATTGGTAACG  
Chattonella: CATTACGCAGGTACTGTTGTAGGTAAGTTAGAAGGTGACCCCTAATGG  
ST4CH31\_clone: TATTCACGCGGGTACTGTAGTAGGTAAGCTTGAAGGGTATCCCTAATGA  
ST1CH4\_clone: TATCCACGCTGGTACAGTTGTAGGTAAGTTAGAAGGAGATCCAAACACAG  
ST3CH27\_clone: TATTCACGCTGGTACTGTTGTAGGTAAGTTAGAAGGTGATCCATTAATGG  
Ectocarpus: TATCCATGCAGGTACTGTAGTTGGTAAACTAGAAGGAGATCCCTAATGG  
Xanthonema: CATTACGCAGGTACTGTTGTTGGTAAATTAGAAGGTGACCCCTTAATGG  
Vaucheria: CATTATGCTGGTACTGTTGTAGGTAAGTTAGAAGGTGACCCCTTAATGG  
Heterococcus: GATCGATT---TAGTTATTGGGTACACAGCCATTCAATGCCATGCTATTT  
Aureoumbra: TATCCACGCAGGTACTGTTGTAGGTAAGTTAGAAGGTGACCCATTAATGG  
Aureococcus: CATTACGCCTGGTACTGTTGTAGGTAAGTTAGAAGGTGATCCATTAATGG  
Pelagococcus: CATTACGCCTGGTACAGTTGTAGGTAAGTTAGAAGGTGATCCATTAATGA  
Cylindrotheca: TATTCACGCTGGTACAGTTGTAGGTAAGTTAGAAGGGGATCCCTTAATGA  
Phaeodactylum: TATCCATGCTGGTACAGTTGTAGGTAAGTTAGAAGGCGATCCCTTAATGA  
ST4CH14\_clone: TATTCACGCTGGTACAGTTGTTGGTAAATTAGAAGGTGATCCCTTAATGA  
ST6CH2\_clone: CATCCACGCTGGTACAGTAGTAGGTAAGTTAGAAGGTGATCCCTTAATGA  
Rhizosolenia: TATCCACGCAGGTACAGTTGTTGGTAAATTAGAAGGTGATCCCTTAATGA  
Skeletonema: CATCCACGCTGGTACAGTTGTTGGTAAAGTTAGAAGGTGATCCCTTAATGA  
Thalassiosira: TATCCATGCTGGTACAGTTGTTGGTAAATTAGAAGGTGATCCCTTAATGA  
Detonula: ACAAGGTTGCTTCTTTCTTTACCTGATTTAACTGACGAACAAATTGAAA  
Chilomonas: CATTATGCTGGTACTGTTGTGGGTAAGTTAGAAGGCGATCCCTTTGATGG  
Mazzaella\_membranacea: TATTCATGCAGGTACTGTTGTTGGTAAACTAGAAGGCGATCCCTAATGA  
Karenia\_brevis: TATTCATGCCGGAACCGTTGTGGGTAAGTTAGAAGGAGATCCAAAAATGA  
Jaxonville\_C3a: TATTCATGCCGGAACCGTTGTGGGTAAGTTAGAAGGAGATCCAAAAATGA  
Mexico\_Beach\_C5: TATTCATGCCGGAACCGTTGTGGGTAAGTTAGAAGGAGATCCAAAAATGA  
Piney\_Island\_A9: TATTCATGCCGGAACCGTTGTGGGTAAGTTAGAAGGAGATCCAAAAATGA  
Charlotte\_Harbor\_C2: TATTCATGCCGGAACCGTTGTGGGTAAGTTAGAAGGAGATCCAAAAATGA  
Charlotte\_Harbor\_A2: TATTCATGCCGGAACCGTTGTGGGTAAGTTAGAAGGAGATCCAAAAATGA

## Appendix 2 (Continued)

Piney\_Island\_B4: TATTCATGCCGGAACCGTTGTGGGTAAACTAGAAAGGAGATCCAAAAATGA  
 Mexico\_Beach\_B3: TATTCATGCCGGAACCGTTGTGGGTAAACTAGAAAGGAGATCCAAAAATGA  
 Appalachicola\_C6: TATTCATGCCGGAACCGTTGTGGGTAAACTAGAAAGGAGATCCAAAAATGA  
 Karenia\_mikimotoi\_1: TATCCATGCCGGAACCGTTGTGGTAAATTAGAAAGGAGAACCTAAAATGA  
 Karenia\_mikimotoi: TATCCATGCTGGAACCGTTGTTGGTAAATTAGAAAGGAGAGCCGAAAAATGA  
     Karlodinium: TTTACATGCTGGAACGGTAGTTGGAAAACTTGAGGGCGATCCTTACATGG  
     Amphidinium: CTACCTCGCAACGGCTGCGCACTTTGCAGCTGAATCATCCACTGGTACCA  
     ST2SY33\_clone: ACTACATACTGTAACCTGTTGTTGGAAAACTAGAAAGGTGATCGTCAAACAA  
     Prochlorococcus: ATTACATACTGGAACCGTGGTTGGAAAACTAGAAAGGTGATCGTCAAACAA  
     Thiobacillus: CCAACACGGGTCTGGCCAACCTGGTGCCGCGACAACGGCATGCTCCTGCAC  
     Synechococcus\_sp: CATCCACACCGGTACCGTTGTTGGTAAAGCTCGAAGGGGATAAAGCCGTTA  
     ST2SY26\_clone: GCTCCACACCGGCACCGTGGTTCGGAAGCTGGAAGGTGATCGTCAGACCA  
     Synechococcus: CCAACACCGGTCTGTCGAAGTGGTGCCGCAAGAACGGCATGTTGCTGCAC  
     Nitrobacter: CCAACGCGGGTCTGGCGAACTGGTGCCGCAACAACGGAAATGCTGCTGCAC  
     Synechocystis: CCTCCACTCCGGTACCGTGGTTGGTAAATTGGAAGGGGAACGGGGTATCA  
 Synechococcus\_PCC7002: CCTCCACTCCGGTACCGTGGTTGGTAAAGCTCGAAGGCGATCGCGCCGCCA  
     ST8SY15\_clone: CCTCCACTCTGGTACTGTGGTAGGAAAGCTCGAAGGTGAAAAAGGCATCA  
     Trichodesmium: CCTCCACTCTGGTACTGTGTCGTTGGCAAGCTCGAAGGA-----  
     ST5SY7\_clone: CTTACATAGTGGTACTGTTGTAGGAAAACTAGAAAGGTGATAAAGATATCA  
     ST2SY2\_clone: CCTTCACTCAGGTACTGTAGTAGGTAAGCTTGAGGGTGAGCGTGAAGTAA  
     ST6SY8\_clone: CCTTCACTCTGGTACTGTAGTAGGTAAGCTTGAGGGTGAGCGTGAAGTAA  
     Volvox\_carteri: GCACGACTACTTAACCTGGTGGTTTTACAGCTAACACATCATTAGCTTCTT  
     Spinacia: TATTCACTCTGGTACCCTAGTAGGTAAGCTTGAAGGAGAAAGAGATATTA  
     Asparagus: TATTCACGCTGGTACAGTAGTAGGTAAGCTGGAAGGGGAACGTGAGATGA  
     Thalassia: GCATGACTACTTAACGGGGGGATTCACTGCAAATACTAGCTTGGCTCGTT  
     Prymnesium: TATCCACGCAGGTACTGTAGTTGGTAAAGCTAGAAAGGTGATCCTCTAATGA  
 Chrysochromulina\_sp: CATTACGCTGGTACTGTAGTAGGTAAGCTAGAAAGGTGATCCTCTAATGA  
     Imantionia: CATCCACGCCGGTACTGTAGTAGGTAAGTTAGAAAGGTGATCCTCTAATGA  
     ST1CH3\_clone: CATTACGCGCGGTACAGTAGTAGGTAAGCTTGAAGGTGATCCACTAATGA  
     Helicosphaera: CATTACGCGAGGTACAGTAGTAGGAAAGTTAGAAAGGTGATCCTCTAATGA  
  
 Emiliana\_huxleyi: TTAAAGGTTTCTACAACACTCTACTTGATACTAAGACTGAAGTTAACCTT  
     ST8CH26\_clone: TTAAAGGTTTCTACAACACTTTATTAGATACTAAGACTGATATTAACCTA  
     Isochrysis: TCAAAGGTTTCTACAACACTCTACTAGATACTAAGACTGATATTAACCTT  
     Calcidiscus: TTAAAGGTTTCTACAACACTCTACTAGATTTCAAGACTGATGTTAACTTA  
 Cruciplacolithus: TTAAAGGTTTCTACAACACTCTATTAGATTTCAAGACTGATATCAATCTG  
     Pleurochrysis: TTAAAGGTTTCTACAACACTCTACTTGACTTCAAGACTGATATTAACCTA  
     Pavlova: TTAAAGGTTTCTATAACACTCTTCTAGAAAACAAAAGTATCAACTTA  
 Exanthemachrysis: TTAAAGGATTCTACAACACTCTACTTGAGAACAAGACTGACATTAACCTA  
     Pyrenomonas: TTAAAGGTTTCTACGATACATTATTAGAAGTTAAAACAGAAGTTAACTTA  
     Chroomonas: TTAAAGGTTTCTACAATACATTACTAGAAGCTAAGACTGATGTTAAACCTT  
     Rhodella: TTAAAGGTTTCTACAACGTTCTATTAGAAACTAGTCTTGACATCAACTTA  
     Dictyocha: TTAAAGGTTTCTATCATACTACTTGATGTTAAGACTGATGTTAAACCTT  
     ST6CH33\_clone: TTAAAGGTTTCTACAACACATTATTAGACTGAAAATAAGATCAACTTA  
     Heterosigma: TATTCGGTTTCAAAGCTGTAGCTGCATTACGTTTAGAAGATATGCGTATT  
     Chattonella: TTAAAGGTTTCTACGACACTTTTACGTGAATGTGAGTTAAGTATCAACTTA  
     ST4CH31\_clone: TTAAAGGTTTCTACAATACACTTTTAGCCACAAAATCAGAGACTTCTCTG  
     ST1CH4\_clone: TTAAAGGATTCTACGATACACTATTATTACCTGCATTAAGAAGATCGT  
     ST3CH27\_clone: TTCGTGGTTTCTATAGAACATTATTAGATAACAGTTTATCTGTTAACTTA  
     Ectocarpus: TTAAAGGATTCTACAACAGTTTATTATTAACCTATTTAAAAATTAATTTA  
     Xanthonema: TTAAAGGTTTCTACAACACTTTTATTACAAAGTGAATCTGAAATCAACTTT  
     Vaucheria: TTAAAGGCTTTTATAATACATTATTATTAAACGAAATTAGAAATTAATCTT  
     Heterococcus: GGGCTCGCCAAGCAGACATGATCTTACATTTACACCGTGCAGGTAACCTT  
     Aureoumbra: TTCAAGGTTTCTACGATACTTTATTATTAACCTCACTTAAAGATTGATTTA  
     Aureococcus: TTCAAGGTTTCTACGATACTTTATTAAAAACTAAGTTAGCAATCGATTTA  
     Pelagococcus: TTCAAGGTTTCTACGACACATTATTAAAGACTAAGTTAGCTATCGATTTA  
     Cylindrotheca: TTAAAGGTTTTTATCATACTTTACGGTTAACAACATTAGATGTTAACTTA

## Appendix 2 (Continued)

Phaeodactylum: TTAAAGGTTTCTACGATACTTTATTACTAACTCATTTAAATGTTAATTTA  
ST4CH14\_clone: TTAAAGGTTTCTACGATACTTTACGTTTAAACAACCTTAGATGTAACTTA  
ST6CH2\_clone: TTAAAGGTTTCTACGATATTTTACGTTTAAACAACCTTAGATGTAACTTA  
Rhizosolenia: TTAAAGGTTTCTACGATGTATTACGTTTACCAGTTTGTAGACGTAACTTA  
Skeletonema: TTAAAGGTTTCTACGATATCTTACGTTTAACTGAATTAGAAGTAACTTA  
Thalassiosira: TTAAAGGTTTCTACGACATCTTACGCTAACTGAATTAGAAGTAACTTA  
    Detonula: AACAAAGTAGCTTGCCTATGTCTAGAGGTTTACGATGAACGTAGAGTGG  
    Chilomonas: TTAAAGGTTTTATATGTTTTACTACGAACCTAAGCTTGATGTAAACCTA  
Mazzaella\_membranacea: TCCGAGGTTTCTATAACACACTACTACTACCATATTTAGAAGTGAATTTA  
    Karenia\_brevis: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Jaxonville\_C3a: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Mexico\_Beach\_C5: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Piney\_Island\_A9: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Charlotte\_Harbor\_C2: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Charlotte\_Harbor\_A2: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Piney\_Island\_B4: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Mexico\_Beach\_B3: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
    Appalachicola\_C6: TTAAAGGTTTCTATGAAACGTTATTGGGTCTGTGTACACGAATTAACCTT  
Karenia\_mikimotoi\_1: TTAAAGGTTTTATAGACATTACTCGATCTAACAACCTAGACATGATTTT  
Karenia\_mikimotoi: TTAAAGGATTCTATAAGACACTCCTGGATTTAACGACCGAACATGATTTT  
    Karlodinium: TCAAAGGTTTTATGCTACTTCTTACAACAAAGGCAGCAGCAAACATA  
    Amphidinium: ACGTGAACGTGTGCACCACCGACTTCACCAAGTCAGTCGATGCACTG  
    ST2SY33\_clone: CTCTTGTTTACATTGACAACCTTAAGAGAGTCATTTGTTCCCGAAGATAGA  
Prochlorococcus: CTCTTGTTTACATTGACAACCTTAAGAGAGTCATTTGTTTCCCGAAGATAGA  
    Thiobacillus: ATTCACCGCCATGCATCGGGTGTGGACCGCAACCCTCACACGGCAT  
Synechococcus\_sp: CCCTCGGCTTTGTGATCTGCTGCGGGAAAACCTACATTGAGCAGGATCGC  
    ST2SY26\_clone: CCCTCGGCTACATCGACCAGCTGCGCGAATCCTTCGTGCCCAGACCGC  
    Synechococcus: ATCCACCGCCATGCACGCGGTGATCGACCGTCAACCCAGCACGGCAT  
    Nitrobacter: ATCCACCGCGCTATGCATGCGGTGATCGATCGCAACCCGCATCACGGTAT  
    Synechocystis: CCATGGGCTTCGTTGACCTCATGCGCGAAGATTACGTTGAGGAAGATCGC  
Synechococcus\_PCC7002: CCCTCGGTTTTCGTAGACCTGATCGGTGAAGACTACGTTGAGGAAGATCGT  
    ST8SY15\_clone: CAATGGGCTTTGTAGACTTAATGCGGGAAGACCATGTTGAGCAAGATCGC  
    Trichodesmium: -----  
    ST5SY7\_clone: CTTTAGGTTTTGTAGACTTAATGAGAGATGATTATGTCAAATTAGACCGT  
    ST2SY2\_clone: CTCTAGGTTTTCGTAGATCTAATGCGTGATAACCTTCAATTGAGAAGGATCGT  
    ST6SY8\_clone: CTCTTGTTTTCGTAGACCTTATGCGTGACGACTTTGTTGAGAAAGGATCGT  
Volvox\_carteri: ACTGTCGTGATAATGGTCTTTTTATTACACATTCACCGTGCATGACACCGA  
    Spinacia: CTTTAGGCTTTGTTGATTTACTACGTGATGATTATATACTGAAAAGACCGA  
    Asparagus: CTTTAGGTTTTGTTGATTTATTACGTGATGATTATATTGAAAAAGACCGA  
    Thalassia: ATTGCCGAGACAACGGATTACTTCTTACATTCACCGCGCAATGCATGCT  
    Prymnesium: TTAAAGGTTTCTACAACACACTTCTAGACTTTAAATCTGAGATGTGCCTA  
Chrysochromulina\_sp: TTAAAGGTTTCTACAACACTCTTCTTGACTTCAAGTCAGATGTAGTCTA  
    Imantionia: TTAAAGGTTTCTACAACACTCTTCTAAACTGTAAAGACTGATGTAAACCTA  
    ST1CH3\_clone: TCAAGGCTTCTACAACACTCTACTTGACTTCAAGACCGACGTAAACCTA  
    Helicosphaera: TTAAAGGTTTCTACAACACGCTTCTAGACTTCAAGACAGATGTAAACTTA  
  
Emiliana\_huxleyi: CCTCAAGGTTTTATTCTTCGCTCAAGATTGGGCTTCACTTCGTAAGTGTGT  
    ST8CH26\_clone: CCTCAAGGTTTTATTCTTCGCTCAGGATTGGGCTTCAATTACGTAAGTGTAT  
    Isochrysis: CCTCAAGGTTTTATTCTTCGCTCAAGATTGGGCTTCTCTTCGTAAGTGTGT  
    Calcidiscus: CCTCAAGGTCTATTTCTTTGCACAAGATTGGGCTTCTTTACGTAAGTGTGT  
    Cruciacolithus: CCTCAAGGTCTATTTCTTCGCGCAAGATTGGGCTTCTTTACGTAAGTGTGT  
    Pleurochrysis: CCTCAAGGTTTTATTCTTCGCGCACAGGATTGGGCTTCTTACGTAAGTGTGT  
    Pavlova: CCGGAAGGTCTATTCTTTGAGCAAGATTGGGCTTCTTTACGTAAGTGTAT  
    Exanthemachrysis: CCAGAAGGTCTATTCTTCGAGCAAGATTGGGCTTCTTACGTAAGTGTAT  
    Pyrenomonas: GTTGAAGGTTTTATTCTTCGCTCAAGACTGGGCTTCTTTAGCTAAGTGTAT  
    Chroomonas: GTTCAAGGTTTTATTCTTCGCTCAAGATTGGGCTTCTTTAGCTAAGTGTAT  
    Rhodella: CCTCAAGGTATATTCTTTGCACAAGATTGGGCTTCTTTACGTAAGTGTAT

## Appendix 2 (Continued)

Dictyocha: CCACAAGGTTTATTCTTCGCCCAAGATTGGGCTTCTCTACGTAATGTCT  
ST6CH33\_clone: CCTCAAGGTATCTTCTTCGATATGGACTGGGCTCTTTACGTCGTTGTG  
Heterosigma: CCTTACGCTTACTTAAAAACATTCCAAGGTCCAGC-----  
Chattonella: CCACAAGGTATCTTCTTGAATGGATTGGGCTCTTTACGTAATGTG  
ST4CH31\_clone: CCCGAAGGCTTATTCTTCGTGCAAGACTGGGCTCTTTACGTAAGTTCGT  
ST1CH4\_clone: ACTTTAGGATTATTCTTCGATATGGATTGGGCTCATTACGTAATGTAT  
ST3CH27\_clone: CCAGAAGGTATTTTCTTCGAAATGGACTGGGCTCTTTACGTAACATT  
Ectocarpus: GCTGAAGGCTTATTCTTTCGATATGGATTGGGCTCTTTACGTAAGTGTG  
Xanthonema: CCTCAAGGTATCTTCTTCGCAATGGATTGGGCTTGTTTACGTAACATT  
Vaucheria: TCTCAAGGTGTATTTTGAATGGATTGGGCTCAGATTACGTAACATT  
Heterococcus: ACATATGCGCGTCAAAAAAATCATGGTATTAACCTCCGTGAATTTGTAA  
Aureoumbra: CCTAAGGCTATTTTCTTCGATATGGATTGGGCTCTTTACGTAAGTGTAT  
Aureococcus: CCTAAGGCTATCTTCTTCGACATGGACTGGGCTGCTTTACGTAAGTGTAT  
Pelagococcus: CCTAAGGCTATCTTCTTCGATATGGACTGGGCTCTTTACGTAAGTGTAT  
Cylindrotheca: CCTTATGGTTTGTCTTCGAAATGTCATGGGCTAGTTTACGTCGTTGTAT  
Phaeodactylum: CCATACGGTATTTTCTTCGAAATGACTTGGGCAAGTTTACGTAATGTAT  
ST4CH14\_clone: CCTTACGGTTTATTCTTCGAAATGGACTGGGCTAGTTTACGTAAGTGTAT  
ST6CH2\_clone: CCATTCGGTATTTTCTTCGAAATGGATTGGGCTAGTTTACGTAAGTGTAT  
Rhizosolenia: CCATTCGGTATTTTCTTCGAAATGGATTGGGCTAGTTTACGTAATGTAT  
Skeletonema: CCTTTTCGGTATCTTCTTCGAAATGGATTGGGCTAGTTTACGTCGTTGTAT  
Thalassiosira: CCTTTTCGGTGTCTTCTTCGAAATGGATTGGGCTAGTTTACGTCGTTGTAT  
Detonula: ACAGATGATCCACATCCTCGTAATAACTACTGGGAATTATGGGTTTTACC  
Chilomonas: CCACAAGGTTGTTCTTTTCGCAAGATTGGGCTTCGCTTGCTAAATGTAT  
Mazzaella membranacea: CCACAAGGTATATCTTTGAACAAGATTGGGCATCTCTTAGAAAAGTTAC  
Karenia brevis: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Jaxonville C3a: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Mexico\_Beach\_C5: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Piney\_Island\_A9: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Charlotte\_Harbor\_C2: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Charlotte\_Harbor\_A2: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Piney\_Island\_B4: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Mexico\_Beach\_B3: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Appalachicola\_C6: AGTCTCGGTTATTTTTCGACAAGAATGGGCTAGTTTACGAAAGTGTG  
Karenia mikimotoi\_1: GCTTTTCGGTCTTTATTTTCGCAAGAATGGGCTTCTCTCCGTAAATGCAT  
Karenia mikimotoi: GCTTACGGTCTTTACTTTTCGCAAGACTGGGCTTCTCTTCGTAATGCAT  
Karlodinium: AAAAAATGGTTTATTTTTCGCAAGATTGGGCTTCGTTGCGCCGATGTG  
Amphidinium: GTGTACTACATCGACCAGAGAATGAGGAGTGAAGATCGCCTACCCAAA  
ST2SY33\_clone: TCAAGAGGTAACCTTCTTTGATCAAGATTGGGGTTCAATGCCAGGATAT  
Prochlorococcus: TCAAGAGGTAACCTTCTTTGATCAAGATTGGGGTTCAATGCCCGGAGTATT  
Thiobacillus: CCACTTCCGTGTGCTGACCAAGATTCTCCGTCTGTCCGGCGGCATCACC  
Synechococcus\_sp: TCCCCTGGTATTTACTTTCACCCAAGACTGGGCTTCGATGCCCGGAGTGTAT  
ST2SY26\_clone: AGCCCGGCAACTTCTTCGATCAGGACTGGGCTCCATGCCCGGCGTGT  
Synechococcus: CCACTTCCGCTTCCTCGCAAGTGTCTGCGTCTGTCCGGTGGTGACCAGC  
Nitrobacter: CCACTTCCGCTATTGACCAAGATCTTGCCTCTGTCCGGCGGCACCAT  
Synechocystis: TCCCCTGGTATTTTCTTCACCCAAGACTATGCCATGCCATGCCATGCCATGCCAT  
Synechococcus\_PCC7002: TCTCGCGGTGATTTCTTCACCCAAGACTACGCTTCTCTCCCCTGGCACCAT  
ST8SY15\_clone: GAACGTGGTATTTACTTTACTCAAGATTGGGCTTCTATGCCCTGGTGTAA  
Trichodesmium: -----  
ST5SY7\_clone: TCAAGAGGTATCTATTTACTCAAGACTGGGCATCAATGGGTGGTGTAA  
ST2SY2\_clone: TCTCGTGGTATTTACTTTACTCAGGATTGGGTATCAATGGGTGGTGTAA  
ST6SY8\_clone: AACCGTGGTACTACTTTACTCAGGAATGGTGTCTCATGGTGGTGTAA  
Volvox carteri: GTAATTGACCGTCAACGTAACCATGGTATTCACTTCCGTGTGTGTACAAA  
Spinacia: AGTCGCGGTATTTATTTCACTCAATCTTGGGTTTCCACACCAGGTGTTCT  
Asparagus: AGTCGCGGTATTTTTTCACTCAAGATTGGGTTTCTATGCCAGGTGTTAT  
Thalassia: GTTATTGATAGACAGAAGAATCATGGTATGCATTTCCGTGTACTAGCTAA  
Prymnesium: GCAGAAGGTCTATTCTTTCGACAAGATTGGGCTTCGCTACGTAAGTGTGT  
Chrysochromulina\_sp: CCTGAAGGTATGTTCTTCGACAAGATTGGGCTTCTCTACGTAAGTGTGT

## Appendix 2 (Continued)

Imantionia: CCTGAAGGTCTATTCTTTGCACAAGATTGGGCTTCTCTACGTAAGTGTGT  
 ST1CH3\_clone: CCTCAAGGTTTATTCTTTGCTCAAGATTGGGCTTCACTACGTAAGTGTGT  
 Helicosphaera: CCTCAAGGTTTATTCTTCGCACAGGATTGGGCTTCACTACGTAAGTGTGT  
  
 Emiliana\_huxleyi: ACCAGTTGCTTCAGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 ST8CH26\_clone: TCCAGTAGCTTCTGG-----  
 Isochrysis: ACCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Calcidiscus: ACCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Cruciplacolithus: ACCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Pleurochrysis: ACCGGTAGCATCTGGCGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Pavlova: GCCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Exanthemachrysis: GCCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Pyrenomonas: GCCAGTTGCTTCAGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Chroomonas: ACCAGTTGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Rhodella: GCCTGTGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Dictyocha: GCCAGTAGCCTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 ST6CH33\_clone: ACCTGTAGCTTCTGG-----  
 Heterosigma: -----AACTGGTATTATT-----GTAGAAGCTGAGCGTCTTGATACA  
 Chattonella: TCCAGTTGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 ST4CH31\_clone: ACCGGTAGCCTCCGG-----  
 ST1CH4\_clone: GCCAGTAG-----  
 ST3CH27\_clone: ACCAGTAGC-----  
 Ectocarpus: TCCGGTAGCTTCTGGGGGA-----ATCCACTGTGGTCAAATGCACCAG  
 Xanthonema: ACCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Vaucheria: ACCTGTAGCATCTGGTGGT-----ATCCACTGTGGACAATACATCAA  
 Heterococcus: GTGGATGCGTATGGCAGGT-----GTGGATCACATTCACGCAGGTACA  
 Aureoumbra: GCCTGTGCTATCGGGTGGT-----ATCCACTGTGGTCAAATGCATCAA  
 Aureococcus: GCCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Pelagococcus: ACCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Cyllindrotheca: GCCTGTGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Phaeodactylum: GCCTGTGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 ST4CH14\_clone: GCCTGTAGCTTACAG-----  
 ST6CH2\_clone: GCCAGTAGCTTCTGG-----  
 Rhizosolenia: GCCTGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Skeletonema: GCCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Thalassiosira: GCCAGTAGCTTCTGGTGGT-----ATCCACTGTGGTCAAATGCACCAG  
 Detonula: ATTATTCGACATTAAGATCTTGCAACAGTAATGTTGAATTAATGAAG  
 Chilomonas: GCCAGTTGCTTCTGGTGGT-----ATCCACTGCGGGCAGATGCATCAA  
 Mazzaella\_membranacea: GCCTGTGCTTCTGGTGGT-----ATCCACTGTGGACAATGCATCAA  
 Karenia\_brevis: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCAGGACAATGCATCAA  
 Jaxonville\_C3a: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Mexico\_Beach\_C5: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Piney\_Island\_A9: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Charlotte\_Harbor\_C2: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Charlotte\_Harbor\_A2: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Piney\_Island\_B4: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Mexico\_Beach\_B3: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Appalachicola\_C6: ACCTGTCGCTAGCGGAGGA-----ATCCACTGCGGGACAAT-----  
 Karenia\_mikimotoi\_1: ACCAGTTGCGAGTGGAGGA-----ATCCACTGCGGGACAAT-----  
 Karenia\_mikimotoi: ACCAGTTGCTAGTGGAGGA-----ATCCACTGCGGGACAATGCATTTT  
 Karlodinium: ACCAGTAGGTTTCAGGTGGT-----ATCCACTGTGGTCAAATGCATAAA  
 Amphidinium: CTTGCTCTTTGACCCGAAC-----ATCCACTGATGG-----  
 ST2SY33\_clone: TGCTGTCGCATCAGGTGGT-----ATCCACTGTCTG-----  
 Prochlorococcus: TGCAGTTCGCATCGGGTGGT-----ATCCACTGTTGGCATATGCCTGCA  
 Thiobacillus: TGCAGTTCGGGTACCGTGGTTCGGCAAGCTCGAAGGCGACCGTGGGCGACC  
 Synechococcus\_sp: GGCCGTGGCCTCCGGTGGG-----ATCCACTGTGGCACAATGCCTGCC  
 ST2SY26\_clone: CGCCGTTGCTTCCGGCGGT-----ATCCACTGTCTG-----

## Appendix 2 (Continued)

Synechococcus: TCCACACCGGCACCGTGGTCGGAAAGCTGGAAGGTGATCGTCAGACCACC  
Nitrobacter: TGCACACCGGCACCGTCGTGGGCAAGCTCGAAGGCGATCGTGCTTCCACC  
Synechocystis: GCCCCGTAGCTTCCGGTGGT-----ATCCACGTATGGCACATGCCCGCG  
Synechococcus\_PCC7002: GCCTGTGGCTTCCGGTGGT-----ATCCACGTATGGCACATGCCTGCC  
ST8SY15\_clone: GCCAGTAGCTTCTGGTGGT-----  
Trichodesmium: -----  
ST5SY7\_clone: GCCAGTTGCTTCAGGTGGT-----  
ST2SY2\_clone: TCCTGTAGCATCAGGTGGT-----ATCCACGTCTG-----  
ST6SY8\_clone: GCCCCGTAGCATCTGGTGGT-----  
Volvox\_carteri: AGCTCTTCGTATGTCTGGT-----GGTGACCACCTTCACTCAGGTACT  
Spinacia: GCCTGTGCTTCAGGCGGT-----ATTCACGTTTGGCATATGCCTGCT  
Asparagus: TCCCGTGGCTTCAGGGGGT-----ATTCATGTTTGGCATATGCCTGCC  
Thalassia: AGCATTACGTATGTCTGGT-----GGGGATCACATTACGCTGGTACG  
Prymnesium: ACCAGTTGCTTCGGGTGGT-----ATCCACTGTGGTCAAATGCACCAA  
Chrysochromulina\_sp: ACCAGTAGCATCTGGTGGT-----ATTCACTGTGGTCAAATGCACCAA  
Imantionia: ACCAGTTGCTTCTGGTGGT-----ATTCACTGTGGTCAAATGCACCAA  
ST1CH3\_clone: ACCAGTAGCTTCTGG-----  
Helicosphaera: ACCAGTAGCTTTCAGGTGGT-----ATCCACTGTGGTCAAATGCACCAA