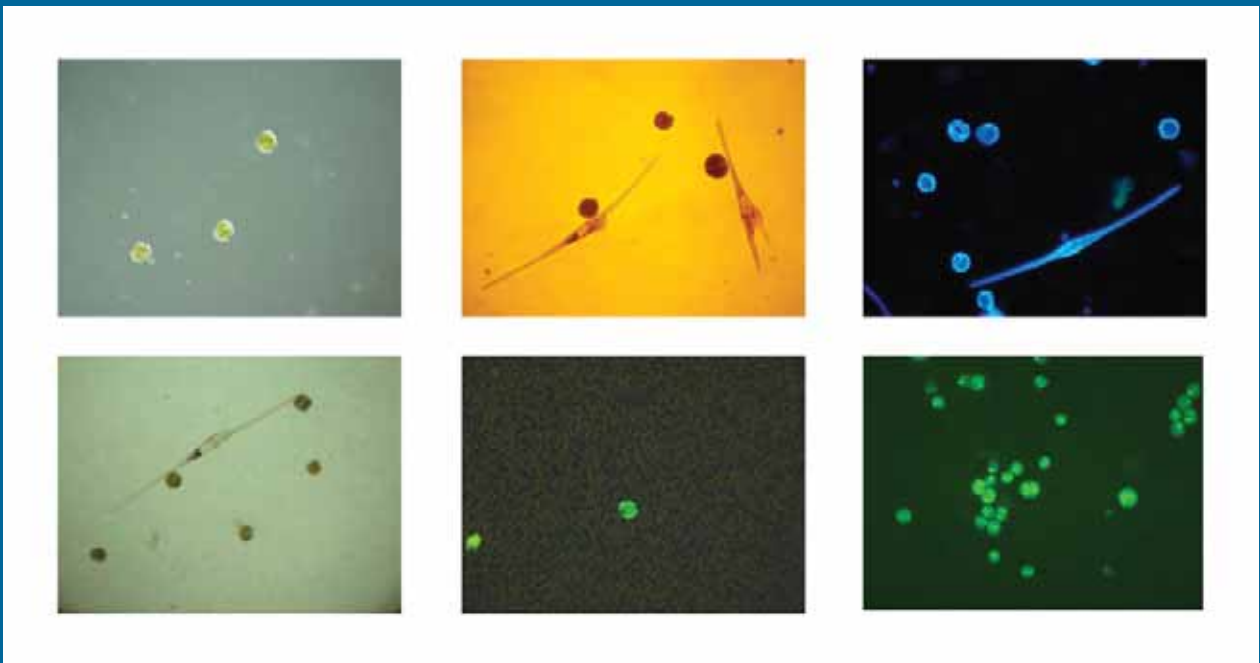




## MICROSCOPIC AND MOLECULAR METHODS FOR QUANTITATIVE PHYTOPLANKTON ANALYSIS



## IOC Manuals and Guides

No.	Title
1 rev. 2	Guide to IGOSS Data Archives and Exchange (BATHY and TESAC). 1993. 27 pp. (English, French, Spanish, Russian)
2	International Catalogue of Ocean Data Station. 1976. (Out of stock)
3 rev. 3	Guide to Operational Procedures for the Collection and Exchange of JCOMM Oceanographic Data. Third Revised Edition, 1999. 38 pp. (English, French, Spanish, Russian)
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9 Annex I	(Superseded by IOC Manuals and Guides No. 17)
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15	Operational Procedures for Sampling the Sea-Surface Microlayer. 1985. 15 pp. (English)
16	Marine Environmental Data Information Referral Catalogue. Third Edition. 1993. 157 pp. (Composite English/French/Spanish/Russian)
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17	Vol. 3: Standard Subsets of GF3. 1996. 67 pp. (English) Vol. 4: User Guide to the GF3-Proc Software. 1989. 23 pp. (English, French, Spanish, Russian) Vol. 5: Reference Manual for the GF3-Proc Software. 1992. 67 pp. (English, French, Spanish, Russian) Vol. 6: Quick Reference Sheets for GF3 and GF3-Proc. 1989. 22 pp. (English, French, Spanish, Russian)
18	User Guide for the Exchange of Measured Wave Data. 1987. 81 pp. (English, French, Spanish, Russian)

*To be continued on page 113*

**MICROSCOPIC AND MOLECULAR METHODS FOR  
QUANTITATIVE PHYTOPLANKTON ANALYSIS**

editors

Bengt Karlson, Caroline Cusack and Eileen Bresnan

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## **Preamble**

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The Intergovernmental Oceanographic Commission of UNESCO has since 1992 given attention to activities aimed at developing capacity in research and management of harmful microalgae. With this IOC Manual & Guide we wish to fill a gap for information and guidance, in an easy accessible and low cost format, to comparison between traditional and modern methods for enumeration of phytoplankton. Enumeration of harmful phytoplankton species is a key element in many monitoring programmes to protect public health, seafood safety, markets, tourism, etc. However, phytoplankton enumeration has self evidently much broader application than just monitoring of harmful microalgae species.

One important task of the IOC and UNESCO is to synthesize the available field and laboratory research techniques for applications to help solve problems of society as well as facilitate further research and especially systematic observations and data gathering. The results include the publications in the 'IOC Manuals and Guides' series, and the UNESCO series 'Monographs in Oceanographic Methodology'. The easy access to manuals and guides of this type is essential to facilitate knowledge exchange and transfer, the related capacity building, and for the establishment of ocean and coastal observations in the framework of the Global Ocean Observing System.

The IOC is highly appreciative of the efforts of the ICES-IOC Working Group on Harmful Algal Bloom Dynamics in organizing the Joint ICES-IOC Intercomparison Workshop on New and Classic Techniques for Estimation of Phytoplankton Abundance at the Kristineberg Marine Research Station in Sweden 2005, and not the least the efforts of the scientists who prepared the manuscripts for this IOC Manual & Guide. The IOC wishes to express its particular thanks to Dr. Bengt Karlson, SMHI Sweden, Editor-in-Chief, for his determination to produce this volume.

The scientific opinions expressed in this work are those of the authors and are not necessarily those of UNESCO and its IOC. Equipment and materials have been cited as examples of those most currently used by the authors, and their inclusion does not imply that they should be considered as preferable to others available at that time or developed since.

The publication of this IOC Manual & Guide has been made possible through support from the United States National Oceanic and Atmospheric Administration and the Department of Biology, University of Copenhagen, Denmark.

Henrik Enevoldsen

IOC Harmful Algal Bloom Programme  
<http://ioc.unesco.org/hab>

## Foreword

Phytoplankton occupy the base of the food web of the sea. It plays a vital role in the global carbon cycle and is also of importance since some phytoplankton may cause harmful algal blooms, a problem e.g. for aquaculture. Man induced changes in the environment, e.g. eutrophication, can be manifested in changes in the phytoplankton community and there is now some evidence that climate change may also be having an effect. Phytoplankton analysis is an essential part in the process of understanding and predicting changes in our environment. Recent introduction of new methods, several based on molecular biology, has led to a perceived need for a manual on quantitative phytoplankton analysis.

The aim of this publication is to provide a guide for phytoplankton analysis methods. A number of different methods are described and information about applicability, cost, training, equipment etc. is included to facilitate information on choosing the right method for a certain purpose. The costs of equipment, consumables, etc. are based on 2009 prices. Although the methods described are for marine plankton they are also applicable for freshwater plankton. The method descriptions are more detailed than what is usually found in scientific articles to make the descriptions useful when setting up monitoring or research programmes that include inexperienced researchers. Some of the methods described are relatively old and well tested while a few must be considered to be emerging technology. We hope that this publication will supplement existing literature and that the distribution of the book freely using the Internet will make it useful in environmental monitoring and for students, researchers and regulators. A book like this can never be complete. Some methods are missing and newer techniques are under development.

The production of this book was initiated during an international workshop at Kristineberg Marine Research Station in Sweden 2005. Participants in the *Joint ICES/IOC Intercomparison Workshop on New and Classic Techniques for Estimation of Phytoplankton Abundance (WKNCT)* agreed to write chapters of the book. A scientific paper describing the results of this workshop can be found in Godhe *et al.* (2007). Co-authors have joined some of the workshop participants. The Harmful Algal Bloom programme of the Intergovernmental Oceanographic Commission, of UNESCO, has aided in the production and also financed the printing of the book. We would like to express our gratitude to everyone who has been involved in the production of this book. In particular the editors would like to acknowledge the time and effort contributed to the final edits and proof reading by Jacob Larsen and Pia Haecky.

Bengt Karlson, Caroline Cusack and Eileen Bresnan

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## 12 Toxic algal detection using rRNA-targeted probes in a semi-automated sandwich hybridization format

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### Introduction

The Sandwich Hybridisation Assay (SHA) provides a simple and rapid means to detect and estimate cell density of a variety of algal species associated with harmful algal blooms (HABs). Results from the SHA system can discriminate to species level using both cultured and natural samples. Having this level of discrimination without the need for microscopy and advanced training in taxonomy, gives researchers, public health officials and water quality managers a powerful tool to rapidly assess changing HAB communities. The SHA system uses species-specific, ribosomal RNA (rRNA) targeted DNA probes that are applied using a semi-automated robotic processor (Scholin *et al.* 1996, 1997, 1999, Greenfield *et al.* 2008). Currently, DNA probes for *Pseudo-nitzschia* spp., *Alexandrium* spp., *Heterosigma akashiwo*, *Chattonella* spp., and *Fibrocapsa japonica* are available (Scholin *et al.* 2004). Others probes include those for *Coccolodinium polykrikoides* (Mikulski *et al.* 2008), a variety of *Karenia* spp., *Karlodinium veneficum* and *Gymnodinium aureolum* (Haywood *et al.* 2007). In New Zealand the SHA method has gained international accreditation and is used to regulate shellfish harvests (e.g. Ayers *et al.* 2005 and references therein). Recent progress made on assays for invertebrates (Goffredi *et al.* 2006), including the invasive European green crab (Jones *et al.* 2008), and marine bacteria (Preston, 2009) offer opportunities for use of the SHA format to detect many other organisms as well.

### Basic Principles of Sandwich Hybridisation

The SHA referred to here (after Scholin *et al.* 1996, 1999, Greenfield *et al.* 2008) employs two DNA probes that target ribosomal RNA (rRNA) sequences. Assays for both the large and small subunit (LSU, SSU) rRNA have been implemented. Assays are performed using pre-filled 96 well microplates and a robotic processor supplied by Saigene Biotech Inc. A capture probe complementary to a variable sequence is attached to a mechanical solid support (prong in a sandwich hybridisation machine), which is then submerged into the prepared sample and hybridizes with the target molecule if present. Captured molecules are then washed to remove any unbound material. To detect the captured molecules, a second hybridisation step is initiated using a DNA probe conjugated to a signal probe. This probe is targeted to a more conserved region of the captured fragment. The resulting “sandwich” of capture probe/target molecule/signal probe is detected using an enzymatically-driven colorimetric reaction. Figure 1 provides a schematic view of the sandwich hybridisation chemistry described above (for details see Greenfield *et al.* 2008).

The basic steps of the SHA method are:

### Universal DNA Probe Based Assay Format

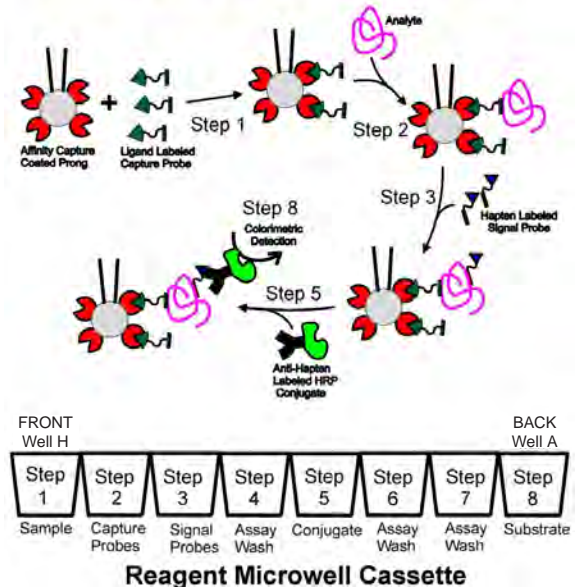


Figure 1. Schematic view of the sandwich hybridisation chemistry

- 1 Collect sample onto a filter,
- 2 Lyse sample using a chaotropic buffer (disrupts and denatures the 3-D structure of macromolecules) and heat,
- 3 Filter lysate,
- 4 Load sample lysate into 96-well plate,
- 5 Run SHA processor (automated),
- 6 Record colour development (O.D. 650 and 450nm),
- 7 Compare results against standard curves to estimate abundance of target species.

### Laboratory Facilities

The SHA system requires a typical laboratory setting that is protected from direct sunlight, excessive dust, and temperature extremes.

#### Essential Equipment

(for more details see Appendix, Table 1 at the end of this chapter)

- Bench top processor (after Scholin *et al.* 1999)
- 96-well Microplate reader that can read wavelengths 650 nm and 450 nm
- Software to record microplate data and apply data conversion algorithm
- Refrigerated storage (2° to 8°C)
- Vacuum filter manifold
- 85 °C heat block
- 12-channel multiple pipette (30-300 µL)

*The fundamentals of*

## **The sandwich hybridisation method**

### **Scope**

Detection and quantification of a target phytoplankton species using ribosomal RNA-targeted, DNA probe-based assays.

### **Detection range**

Detection performance is unique to each probe set used in the SHA system.

### **Advantages**

The SHA system provides a robust and simple semi-automated method to detect and estimate cell abundances of target species.

### **Drawbacks**

Probes are only available for a limited number of target species. Specificity of probes must be established on a regional basis. This system may not be suitable for detection of very rare target sequences.

### **Type of training needed**

Instruction in setting up this technique should come from a person with an in-depth knowledge and experience of molecular biology. A minimum of three days are needed for to cover theory, operation, data processing, etc. A skilled molecular biologist should be available to solve any problems that may arise using this method.

### **Essential Equipment**

SHA semi-robotic processor, microplate reader, heating block, filtration manifold, 12-channel and single channel micropipetors.

### **Equipment cost\***

SHA semi-robotic processor, €5139 (US \$7500)  
Total set-up cost = €14197 (US \$20666).  
See Appendix, Table 1 for details

### **Consumables, cost per sample\*\***

€5-7 (US \$7-10).

### **Processing time per sample before analysis**

15-20 minutes (hands-on)

### **Analysis time per sample**

75 minutes (hands-off).

### **Sample throughput per person per day**

30-40 samples in an 8 hour day given 1 processor.

### **No. of samples processed in parallel**

6, 8, or 12 samples with replicates 4, 3, or 2, respectively.

### **Health and Safety issues**

Relevant health and safety procedures must be followed. The lysis and signal probe buffers contain guanidine thiocyanate, which can damage skin and eyes.

\*service contracts not included

\*\*salaries not included

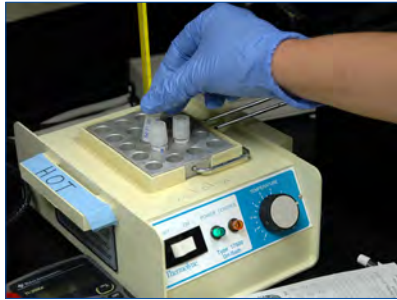


Figure 2. Heating block set to 85°C used for sample lysis.



Figure 3. Three-position vacuum filter manifold used to collect particulate fraction of water sample.



Figure 4. Filter, sample side in, being placed in cryovial for archival and/or lysis.



Figure 5. Addition of lysis buffer to cryovial with filter.



Figure 6. After lysis, lysate is syringe filtered into clean tube.

- 100-1000 µL single channel pipette
- Cold storage for samples: liquid nitrogen is preferred, -80°C and -20°C (e.g., freezer or dry ice) can also be effective for storing archival samples (filters)
- 

#### Chemicals and Consumables

(for more details see Appendix, Table 2 at the end of this chapter)

- 25mm hydrophilic Durapore filter
- 2mL cryovial
- 13mm syringe filter
- 5cc syringe
- Polypropylene tubes 12X 75mm
- 10% H<sub>2</sub>SO<sub>4</sub>
- filling trays/pipette tips

#### Probes

The suite of SHA probes currently available for HAB spp. include the following:

#### Diatoms

*Pseudo-nitzschia australis* (and other diatoms below, Scholin *et al.* 1999)

*Pseudo-nitzschia multiseriis*

*Pseudo-nitzschia pungens*

*Pseudo-nitzschia pseudoelaticissima/multiseriis* complex

#### Dinoflagellates

*Alexandrium tamerense/catenella/fundyense* (North American ribotype, Matweyou *et al.* 2004, Anderson *et al.* 2005)

*Cochlodinium polykrikoides* (Mikulski *et al.* 2008)

*Gymnodinium aureolum* (and other dinoflagellates below, Haywood *et al.* 2007)

*Karenia brevis*

*Karenia mikimotoi*

*Karenia selliformis*

*Karenia papilionacea*

*Karlodinium veneficum*

#### Raphidophytes

*Heterosigma akashiwo* (and others below, Tyrrell *et al.* 2001, 2002, Scholin *et al.* 2004)

*Fibrocapsa japonica*

*Chattonella antiqua/subsalsa*

#### Method

##### Preparation to run samples

- 1 Turn on heating blocks for sample lysis (Fig. 2) and processor and check that desired temperatures are at their proper values. Lysis is carried out at 85 °C. The processor plate should provide a temperature of 28-30 °C.
- 2 Obtain necessary lysis tubes for runs or alternatively label 2 mL cryovials for storage of sample filters in liquid nitrogen for later analysis (see below).
- 3 Start the microplate reader.
- 4 If using pre-made plates, remove seal and let it reach room temperature protected from light and dust. If required, dispense reagents into 96-well microplate as shown in the instruction booklet that comes with materials supplied by Saigene Corporation (see also Greenfield *et al.* 2006). When dispensing reagents to a microplate use barrier tips

to minimise cross-well contamination. Dispense 0.25 mL per well. Do not blow out small amounts of fluids following primary delivery of reagents and samples to the plate or excessive bubbles will form in the well; bubbles can interfere with the assay.

### Sample, plate and prong handling

- 5 Protect plate and sample from sunlight and excessive heat. Samples should be filtered and lysed as soon as possible, or filters can be archived for later analysis by rolling the filter into a cryovial (particles away from the tube wall and freezing the filters in liquid nitrogen or alternative cold storage). Use plate within 1 hour after removing seal and/or dispensing reagents. Prongs should remain in package until used. Handle prongs with forceps touching only the strip, or backbone, that connects the 12 prongs; avoid touching the prongs themselves. Store prongs at 4-8 °C with packaging and desiccant provided.

### Sample filtration

- 6 Samples should be collected using a vacuum manifold at a vacuum pressure of approximately 100-150 mmHg (Fig. 3). Filter samples onto hydrophilic Durapore filters (generally 0.65-0.45 µm pores size; Millipore). The volume filtered should be no more than what can pass through the filter in about 20 minutes. Typical sample volumes are 200 to 400 mL of whole water. Samples that have been pre-concentrated (e.g. net tow or sieve) can also be collected on the Durapore filter as well.

### Archival and Lysis of Sample

- 7 After filtering, place filter membrane into a 2 mL cryovial. Place the filter with the sample side facing away from the tube wall. Do not crumple the filter and be sure to push the filter to bottom of the tube (Fig 4). If sample is to be archived, cap the 2 mL cryovial and store in liquid nitrogen without lysis buffer.
- 8 To process, add 1-2 mL of lysis buffer to cryovial with filter and cap it tightly (Fig 5). Place cryovial in heating block with wells half filled with water to enhance heat transfer (Fig. 2). Heat for 5 minutes total with a brief finger vortex after 2.5 minutes.
- 9 After heating, allow lysate to cool for 5 minutes. Use lysate within 20 minutes of preparation.
- 10 Remove plunger from a 5 cc disposable syringe (Becton and Dickinson).
- 11 Install a 13 mm, 0.45 µm Millex-HV (Millipore) filter onto the syringe.
- 12 Place tip into a clean polypropylene collection tube, add lysate to syringe barrel and push lysate through filter until foam appears at the tip of filter (Fig. 6). Lysates from replicate samples may be combined to yield a larger volume of lysate from a given sample.
- 13 The lysate is now ready to be loaded onto the plate sample well (row H).

### Processing

- 14 Load 0.25 mL filtered lysate per sample well (Fig. 7).
- 15 Load prong onto processor arm and secure spring clip. Do not handle prong with bare hands. Hold prong by the backbone with tweezers and avoid scraping the prongs against the processor arm.



Figure 7. Lysate (0.25 mL) is added to appropriate sample wells on microplate.



Figure 8. Microplate being placed on processor after prongs added to processor arm.



Figure 9. Processor being started after plate is positioned on heater base.



Figure 10. Microplate is placed on microplate reader cradle; optical density is recorded at 650nm for row "A" (wells in which colour development has occurred).



Figure 11. After microplate is read at 650nm, row "A" is acidified with 50 µL of 10% H<sub>2</sub>SO<sub>4</sub>; optical density is recorded at 450 nm for row "A". The measure at 650 nm is considered low sensitivity while that at 450 nm is considered high sensitivity. For positive samples, the optical density at 450 nm should be roughly 2X that at 650 nm.

- 16 Mount 96 well microplate onto processor, make sure to centre the microplate on the heating plate (Fig. 8).
- 17 Push the "RUN" button (Fig. 9) and make sure that the prongs enter the wells without touching the sides of the wells. The plate will be ready to read in just over an hour. A digital timer will display time remaining; the counter will read "0" approximately 90 seconds before the process is actually complete. The processor will display a message indicating that the reaction is complete, and plate is ready to be read.

#### Reading the Plate

- 18 When the processor is finished, immediately read the plate(s) (Fig. 10).
- 19 First read the plate at 650 nm.
- 20 Next acidify row A with 50  $\mu\text{L}$  of 10 %  $\text{H}_2\text{SO}_4$  (add and mix using a 12-channel pipette and avoid introduction of bubbles) (Fig. 11).
- 21 After all bubbles have popped (~10-45 seconds) read plate at 450 nm.

#### Data Processing

To calculate the concentration of target in a sample, the assay requires that you establish an empirically derived dose response curve that relates optical density (O.D.) to a known number of cells per well of lysate (see Greenfield *et al.* 2008, Haywood *et al.* 2007, Ayers *et al.* 2005 for details in establishing a dose response curve). Using optical density (from the assay), the dose response curve, sample volume and lysis buffer volume (used to lyse the sample) you can estimate the target abundance in the sample. Below is the equation used to convert cells per well to cells per mL.

*Cells per mL in sample =*

$$\left(\frac{\text{cells}}{\text{well}}\right) \times \frac{\text{well}}{0.25 \text{ mL lysate}} \times \left(\frac{\text{filter}}{X \text{ mL sample}}\right) \times \frac{Y \text{ mL lysate}}{\text{filter}}$$

Here is an example of how you use the above equation to estimate target abundance in a sample. You filter 500mL (X) of water onto a filter and lyse this sample in 2.0 mL of lysis buffer (Y). Using the SHA system you obtain an O.D. that translates to 625 cells per well (from the dose response curve). Then using the formula you estimate a target abundance of 10 cells per mL in your sample.

$$\begin{aligned} \left(\frac{625 \text{ cells}}{\text{well}}\right) \times \frac{\text{well}}{0.25 \text{ mL lysate}} \times \left(\frac{\text{filter}}{500 \text{ mL sample}}\right) \times \frac{2 \text{ mL lysate}}{\text{filter}} \\ = \frac{10 \text{ cells}}{\text{mL of sample}} \end{aligned}$$

#### Controls

Positive and negative controls are available to check system integrity and performance (e.g. Greenfield *et al.* 2006).

#### Sample Collection and Preservation

Samples should be run or archived as soon as possible after collection. Prior to filtration, samples should be kept cool and

protected from excessive light. If the sample cannot be run within several hours it should be filtered and stored frozen. A sample stored in liquid nitrogen can be held for up to 1 year or longer. Samples can also be stored in a -80 °C freezer or on dry ice for up to 1 week. It may be possible to store preserved samples at room temperature as well (see Tyrrell *et al.* 2002).

## Discussion and system considerations

The goal of SHA system is to give the research/monitoring community a method to quickly and conveniently screen samples for a variety of HAB species. Once samples are collected, processing takes approximately 1.25 hours. No target amplification is required so problems that can affect amplification based systems (e.g. extensive sample handling, PCR inhibition) are avoided. The absolute detection level of the SHA system is dependant on the designs of the capture and signal probes. An important feature of the SHA system is that it is relatively insensitive to biomass, so techniques such as sieving to collect large volumes of sample can be used without impacting system performance, provided proper control experiments have been performed to verify assay results from a given region and wide range of samples. Further increases in sensitivity can also be achieved by lysing the sample in a smaller volume of lysis buffer to increase target cell concentration. The SHA system is not suitable for very rare targets (e.g. single copy genes); in those cases some kind of amplification technique may be desirable.

All methods relying on molecular probes for detection can be subject to cross-reaction with non-target species. Therefore after an initial positive result, an alternative method (microscopy, toxin detection, PCR, etc.) should be used to confirm results until such time that confidence in the efficacy of probe is known. Some probes may work well for certain species in certain regions, but not all probes will work equally well in different geographic regions. Moreover, species designations as defined using traditional criteria (morphology, ultrastructure, pigments, etc.) may not agree with those based on rRNA sequence identity (e.g. see Scholin *et al.* 2004, Ayers *et al.* 2005, Lundholm *et al.* 2006). Provisions should also be made to store replicate samples in case of system failure or if results require further analysis or reconfirmation is desired.

Use of the SHA system requires that probes be available for the target species of interest. Currently, probes are available for a variety of HAB spp. (see above) as well as other organisms. The creation of probes for this system often requires iterative probe design (trial and error) which can incur considerable time and expense. Ideally, cultures of the targeted species are used to create calibration curves and to spot check the system when reagent batches are changed.

The SHA system uses chemistry that is designed to work at 30 °C. If the ambient temperature exceeds this value, the system will not function properly and steps to lower ambient temperature will need to be taken. Some of the reagents used in the SHA system are required to be refrigerated and protected from direct sunlight.

#### Acknowledgements

Development and application of the SHA for HAB research have been sponsored in large part by grants from the David and Lucille Packard Foundation (allocated by the Monterey Bay Aquarium Research Institute), the National Science Foundation (9602576 and OCE-031422), and the National Oceanic Atmospheric Administration Saltonstall-Kennedy Grant Program (NOAA NA57D009) to C.A.S.

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## Appendix

Table 1. Equipment and suppliers. Note that some pieces of equipment, such as the plate reader, sample filtration system, heating block and refrigerator, are available as other models from a variety of vendors. Prices quote obtained December, 2007.

Equipment	Supplier	Cat. Number	€	US \$
96-well Microplate reader that can read wavelengths 650 nm and 450 nm	Fisher Scientific	14-386-27	3821	5510
Optical filter 650nm	Fisher Scientific	14-386-59	196	283
Vacuum filter manifold	Fisher Scientific	09-753-39A	540	779
25 mm polysulfone filter funnel (250 mL) need 6, costs in total	Pall	4203	504	726
Vacuum pump	GAST	DOA P704 AA	303	443
Heating block	Fisher Scientific	11-716-68Q	223	322
12-Channel Pipettor 20-300 $\mu$ L	Rainin	L12-300	412	595
Single Channel Pipettor 10-1000 $\mu$ L	Rainin	PR-1000	184	265
Robotic processor	Saigene Inc <sup>s</sup> .	6000-01	5708	8500
Refrigerator (4-8°C)	Maytag	MBB1952HE	577	850
Cryogenic Storage vessel	Fisher Scientific	11-676-1C	2350	3393
<b>Sum approx.</b>			<b>14818</b>	<b>21666</b>

Table 2. Expendable reagents and supplies required for application of the SHA and relevant suppliers.

Material	Supplier	Cat. Number	€	US \$
<sup>1</sup> Custom Plate One Probe Set; minimum order of 50 plates	Saigene <sup>5</sup>	call for order	25	38 per plate
<sup>1</sup> Custom Plate Two Probe Set; minimum order of 50 plates	Saigene <sup>5</sup>	call for order	27	40
<sup>1</sup> Custom Plate Three Probe Set; minimum order of 50 plates	Saigene <sup>5</sup>	call for order	28	42
<sup>1</sup> Custom Plate Four Probe Set; minimum order of 50 plates	Saigene <sup>5</sup>	call for order	29	44
<sup>2</sup> Assay Development Kit; minimum order of 25 plates	Saigene <sup>5</sup>	call for order	24	36
<sup>3</sup> Bulk lysis buffer, 500 mL	Saigene <sup>5</sup>	call for order	27	40
<sup>4</sup> Defined Kits (routine production), incl lysis buffer, prong, sample filters, etc.			Call for quote	Call for quote
25 mm Durapore filter (100 count)	Millipore	DVPP02500	60	86
13 mm syringe filter (100 count)	Millipore	SLHVT13NL	138	197
Polypropylene 12X75mm tubes (5000 count)	Fisher Scientific	14-961-11	318	459
5 cc Syringe (400 count)	Fisher Scientific	14-823-35	47	68
2 mL Cryovial (250 vials)	Fisher Scientific	03-337-7H	127	184
Filling boats (200 count)	Fisher Scientific	07-200-127	76	110
10% H <sub>2</sub> SO <sub>4</sub> (dilute stock)	Fisher Scientific	A300-500	40	58
<b>Sum approx.</b>			<b>966</b>	<b>1402</b>

<sup>1</sup>Plates made custom to user specification. Saigene will fill and seal plates; user supplies capture and signal probes. Plates are configured with one to four probe sets. Price includes prongs; lysis buffer sold separately. Discount available for orders >50 plates.

<sup>2</sup>Plates filled with all reagents except capture and signal probes (to facilitate assay development). Price includes prong, buffers for preparing capture and signal probe solutions. Discount available for orders >25 plates.

<sup>3</sup>In addition to lysis buffer, Saigene can provide bulk quantities of other reagents used in the SHA; prices available on request.

<sup>4</sup>Defined Kits are those prepared entirely by Saigene. They differ from Custom Plate configurations in that Saigene provides user-defined probes, or those available through published articles. Defined Kits also include prongs and lysis buffer (volume based on the intended use of the plates), and can be bundled with sample and lysate filters if desired. The most likely application of Defined Kits are for research/monitoring programs where there is a defined set of target species/probes, sample and lysis volumes are fixed within well specified range, and users prefer not to take responsibility for procurement and quality assurance of probe stocks. Depending on the number of plates ordered, Defined Kits will generally exceed those in cost of the equivalent Custom Plate by 10-20% depending on probe costs and number of probe sets required per plate.

<sup>5</sup>Saigene Biotech Inc.  
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## Appendix: Acronyms and Notation

Acronym	Meaning
ABD	Area-Based spherical Diameter
ADPA	N-Phenyl-1,4-benzenediamine hydrochloride
ALGADEC	Development of a RNA -Biosensor for the Detection of Toxic Algae
approx.	Approximately
ARB	“arbor ”=tree
BSA	Bovine Saline A
CCD	Charge Coupled Device
CEN	Comité Européen De Normalisation
ChemScan™	Lazer scanning Process Analyser/solid-phase cytometry (Scan RDI™ in North America)
Chl a	Chlorophyll a
CICEET	Cooperative Institute for Coastal and Estuarine Technology
CPR	Continuous Plankton Recorder
CTD	Conductivity, Temperature, Depth
dH <sub>2</sub> O	distilled water
DAPI	4',6-diamidino-2-phenylindoline
DIC	Differential interference contrast
DICANN	Dinoflagellate Categorisation by Artificial Neural Network
DSP	Diarrhetic Shellfish Poisoning
ECOHAB	The Ecology and Oceanography of Harmful Algal Blooms
EPA	Environmental Protection Agency
ESD	Equivalent Spherical Diameter
ESP	Environmental Sample Processor
EU	European Union
EU DETAL	Rapid and ultra-sensitive fluorescent test for the tracking of toxic algae in the marine environment
FISH	Fluorescence <i>In Situ</i> Hybridisation
FIT	Fluid Imaging Technologies
FITC	Fluorescein isothiocyanate
FlowCAM	Flow Cytometer And Microscope
FSW	Filtered Sea Water
FTF	Filter-Transfer-Freeze
GF/F	Glass Fibre Filters
HAB(s)	Harmful Algal Bloom(s)
HAB Buoy	Harmful Algal Bloom Buoy
HAE(s)	Harmful Algal Event(s)
Hybe	Hybridisation
ICES	International Council for the Exploration of the Sea
IOC	Intergovernmental Oceanographic Commission of UNESCO
LED	Light-Emitting Diode
LM	Light Microscopy
LSU	Large SubUnit
MBARI	Monterey Bay Aquarium Research Institute
NASA	National Aeronautics and Space Administration
NOAA	National Oceanic and Atmospheric Administration
NSF	National Science Foundation
ONR	Office of Naval Research
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
PICODIV	Picoplankton Diversity
PSP	Paralytic Shellfish Poisoning
QC	Quality Control
rDNA	Ribosomal Deoxyribonucleic Acid
RNases	Ribonucleases
rRNA	Ribosomal Ribonucleic Acid
RT-PCR	Real-Time PCR Please note that RT-PCR usually refers to reverse transcriptase PCR, In this manual the acronym refers to Real Time PCR.
SEM	Scanning electron Microscopy
SOP	Standard Operating Procedure
SSU	Small SubUnit
TEM	Transmission Electron Microscopy
TSA-FISH	Tyramide Signal Amplification has been used with FISH
UNESCO	United Nations Educational Scientific and Cultural Organisation
wt/vol	weight/volume

<b>SI Unit</b>	<b>Meaning</b>
I <sub>2</sub>	Iodine
KI	Potassium Iodide
mL	millilitre
L	Litre
cm	Centimetre
hr	Hour
°C	Degree Celsius
%	Percentage
µm	Micrometre
mm <sup>2</sup>	Millimetre squared
Min <sup>-1</sup>	Per minute
Hg	Mercury
n	Number
g	Gram(s)
µL	Microlitres
v/v	Volume to volume
x g	Multiplied by gravity



The Intergovernmental Oceanographic Commission (IOC) of UNESCO celebrates its 50th anniversary in 2010. Since taking the lead in coordinating the International Indian Ocean Expedition in 1960, the IOC has worked to promote marine research, protection of the ocean, and international cooperation. Today the Commission is also developing marine services and capacity building, and is instrumental in monitoring the ocean through the Global Ocean Observing System (GOOS) and developing marine-hazards warning systems in vulnerable regions. Recognized as the UN focal point and mechanism for global cooperation in the study of the ocean, a key climate driver, IOC is a key player in the study of climate change. Through promoting international cooperation, the IOC assists Member States in their decisions towards improved management, sustainable development, and protection of the marine environment.