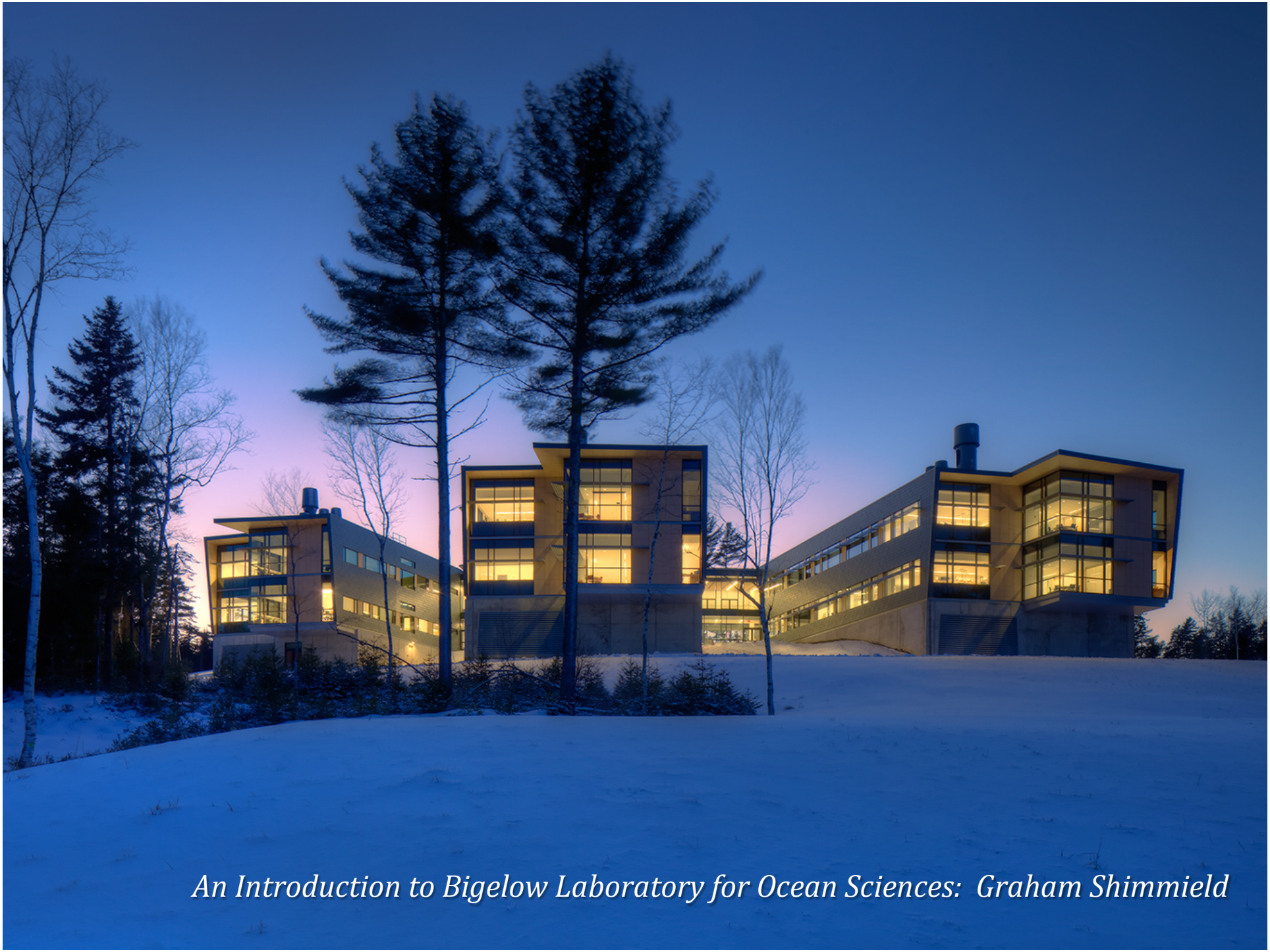
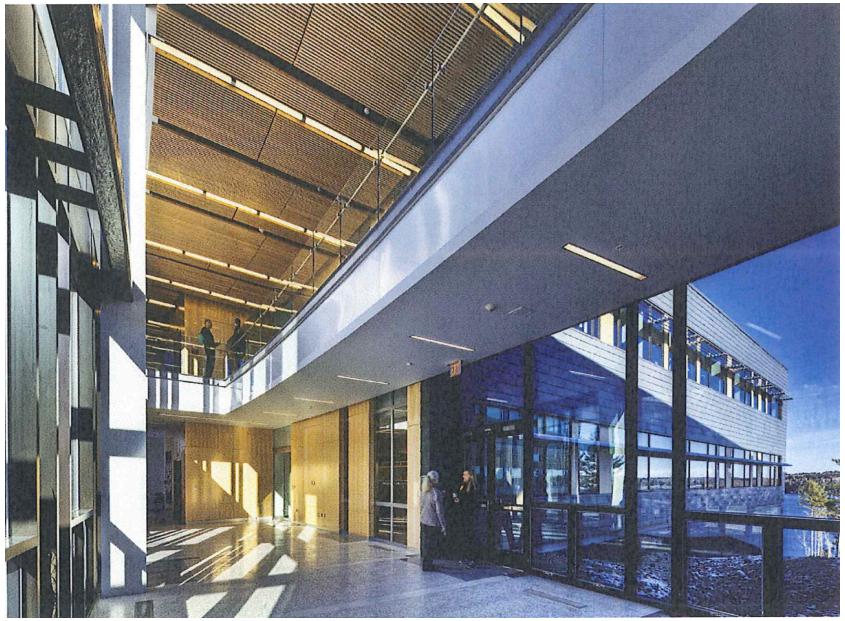
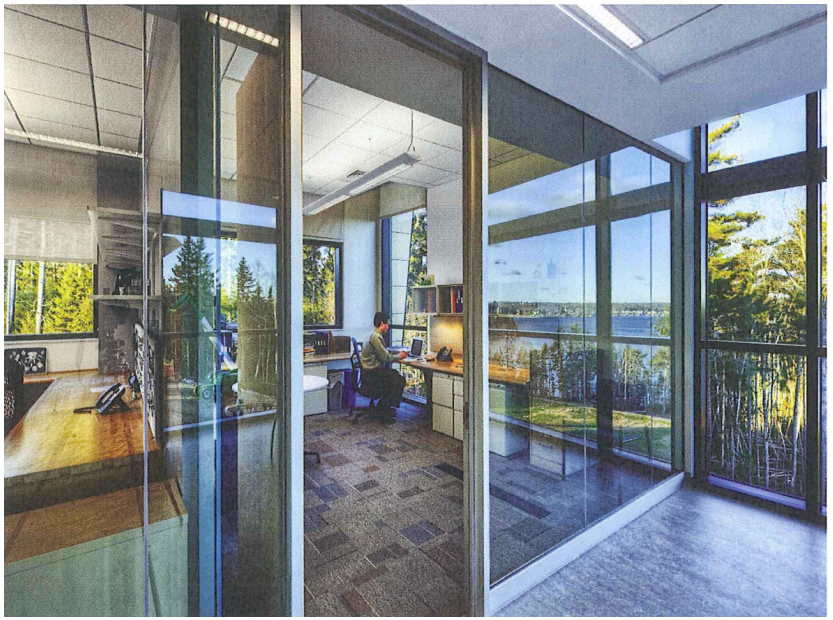
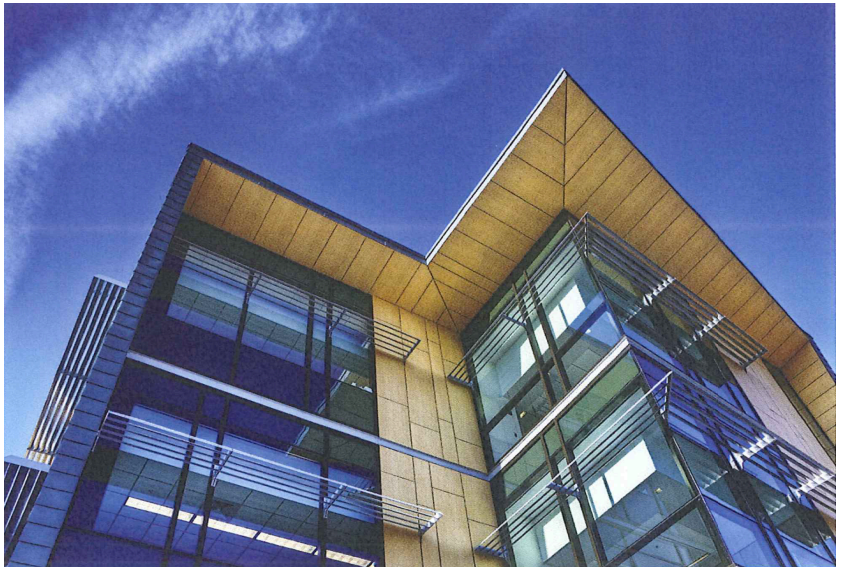
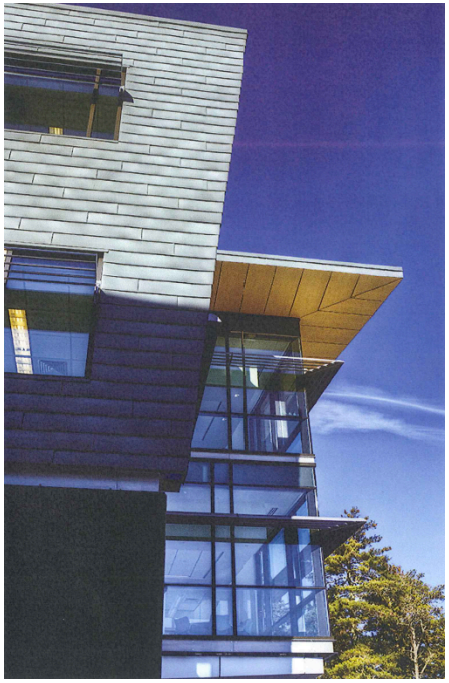

An Introduction to Bigelow Laboratory for Ocean Sciences: Graham Shimmield

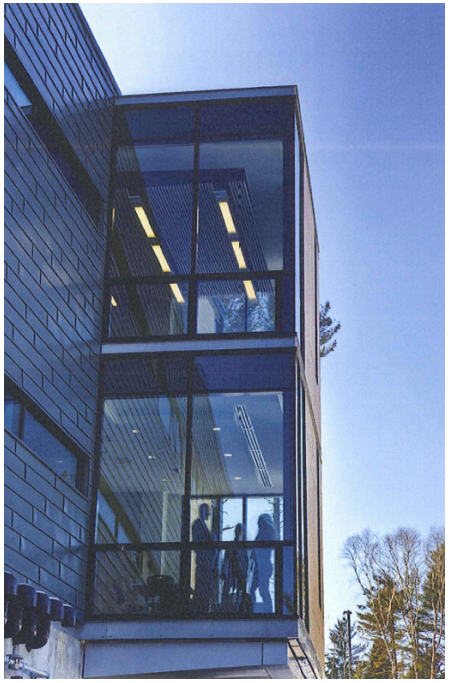




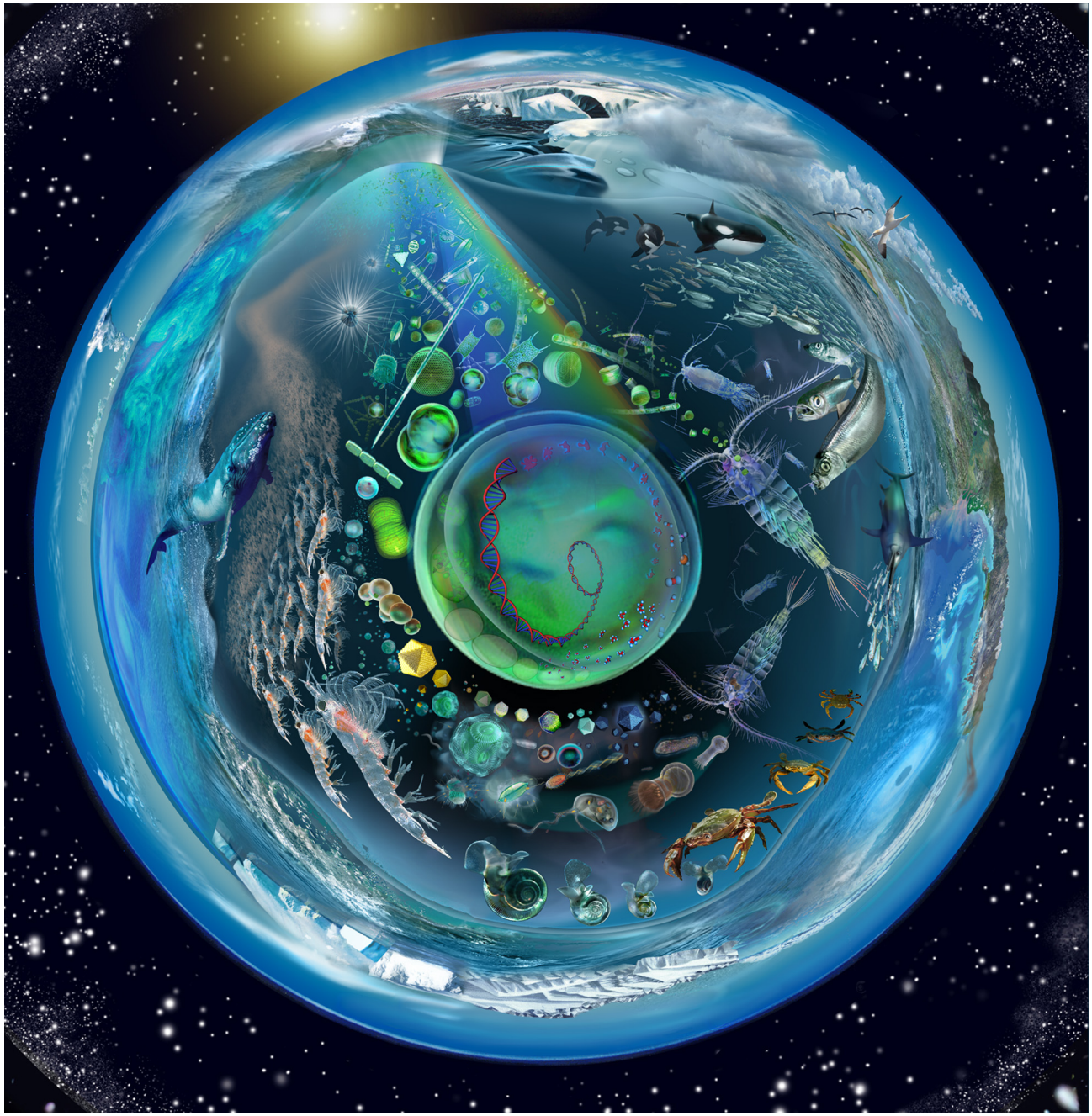


Dec 2012

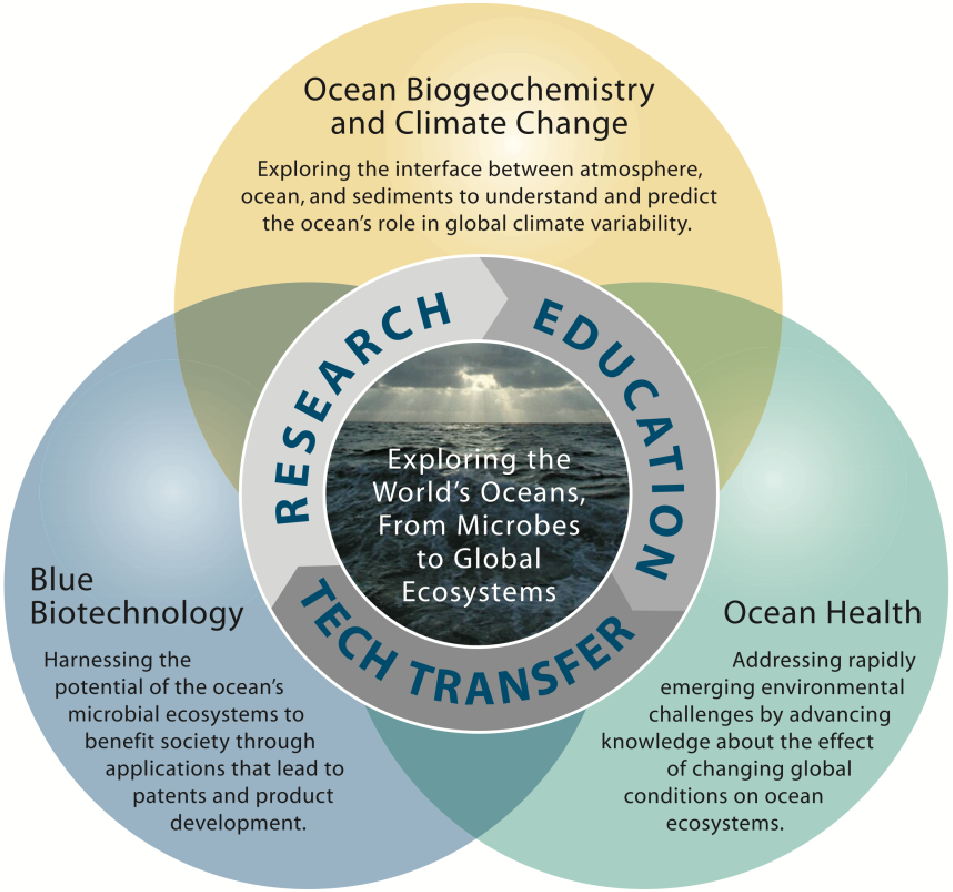





Shore Facility



Core Centers of Discovery: addressing ocean issues in the 21st century

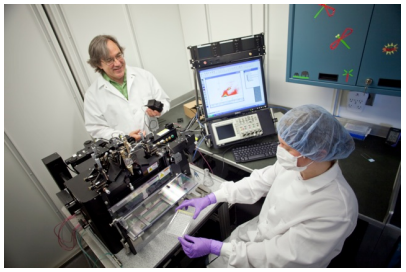


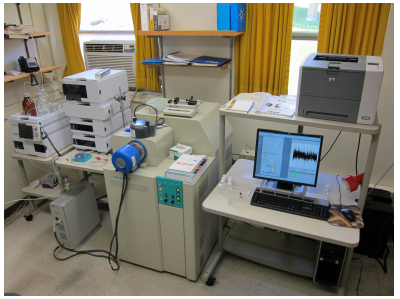
Core Facilities

- Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA)
- J.J. MacIsaac Facility for Aquatic Cytometry
- Single Cell Genomics Facility (SCGC)
- Bigelow Analytical Services (BAS)
- Seawater Facility

Core Service Centers:

- National Center for Marine Algae and Microbiota
- Single Cell Genomics Center
- Aquatic Flow Cytometry Center
- Bigelow Analytical Services
- Seawater Mesocosm and Large Scale Algal Culture Facility


PROVASOLI-GUILLARD
NCMA
National Center for Marine Algae and Microbiota

BIGELOW LABORATORY FOR OCEAN SCIENCES

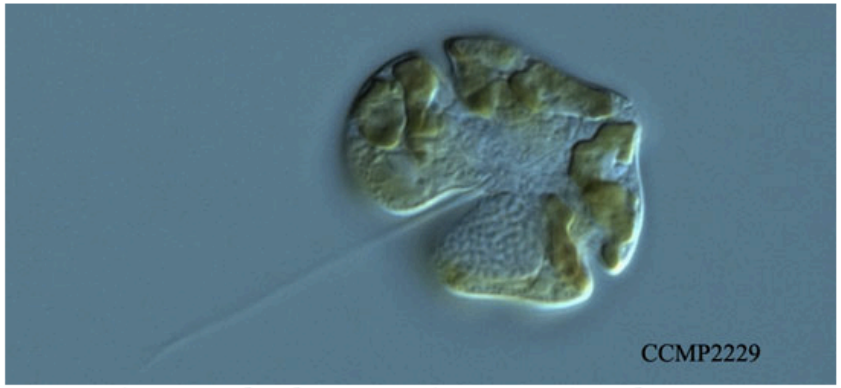
NCMA Site Search
powered by Google

Quick Links

- Search Algae
- Search Bacteria & Archaea
- How to Order
- Depositing Strains
- Aquaculture Express Strains
- Algal Medium Recipes
- Bacterial Medium Recipes

Catalog

- Medium Kits (12)
- Prepared Medium (17)
- Seawater (5)
- Algal Strains (2725)
- Bacterial Strains (74)



NCMA (Formerly the CCMP) - A National Center

We have moved! Please note our new address:

60 Bigelow Drive, PO BOX 380, East Boothbay, Maine 04544
Phone: (001) 202 747 3255 x202
Fax : (001) 202 747 3258

The NCMA is the national marine phytoplankton collection, now incorporating bacteria and viruses, and it is an integral part of Bigelow Laboratory for Ocean Sciences. The NCMA maintains over 2700 strains from around the world, most are marine phytoplankton but we also have benthic, macrophytic, freshwater and heterotrophic organisms.

You can search our online catalog for strains using taxonomic, geographic and other parameters. Strain records have (when available):

- collection and isolation information

Latest News & Announcements

Vote for Willie Wilson to the Algal Biomass Organization (ABO) Board of Directors

"In my role as Director of the NCMA I have an excellent overview of the algal industry through my interactions with a wide range of industry and academic clients, all interested in the basics of growing algae for their respective ventures"

NCMA: A Snapshot

- The world's largest and most diverse living archive of marine microalgae.
- 35 years in the algae business.
- Started as a algal seed stock for the aquaculture industry.
- A repository for public and private collections of algae.
- 2,720 strains of marine, brackish, hypersaline and freshwater algae; including cyanobacteria and macroalgae.
- 359 genera and 723 species.
- 6 products available for every strain.
- 5 growth temperatures (polar to tropical).
- On-site & off-site back-up and cryopreservation.
- Expansion to include new Bacteria and Virus collections.
- New web site to enhance customer experience (Oct 2013).

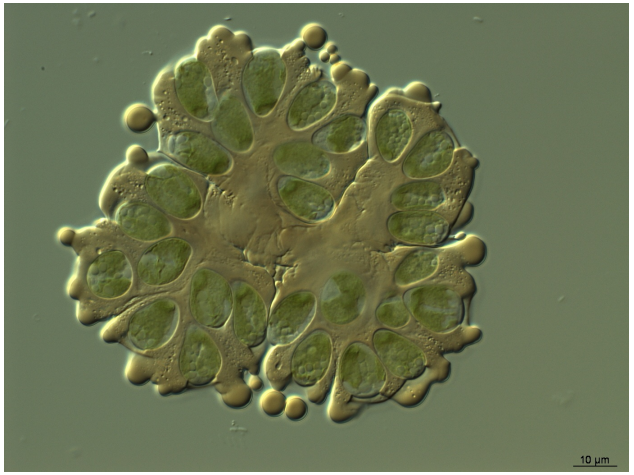
PROVASOLI-GUILLARD NCMA
National Center for Marine Algae and Microbiota
Culturing Diversity

BIGELOW LABORATORY got algae? bigelow.org FOR OCEAN SCIENCES

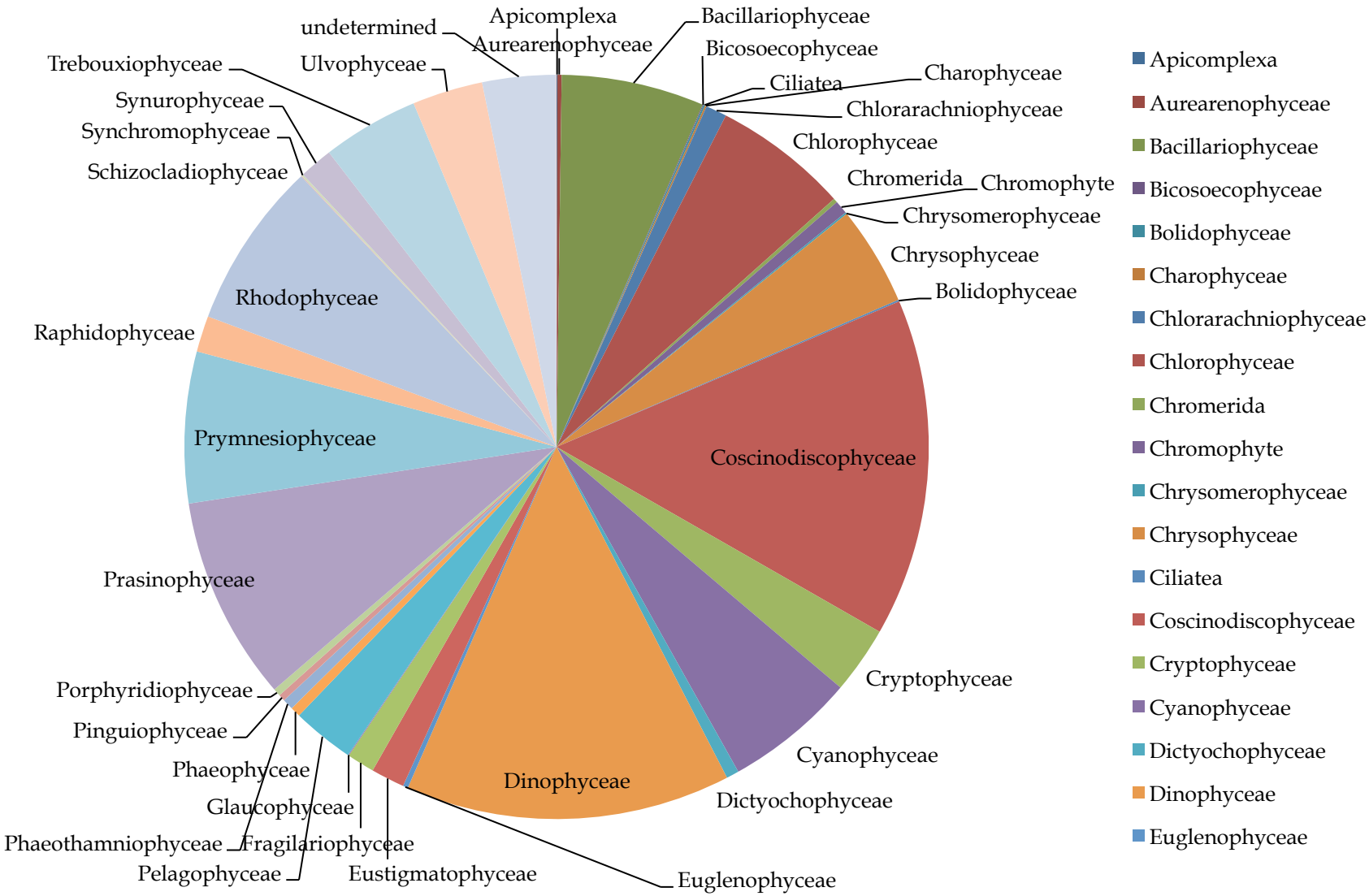
BIGELOW LABORATORY FOR OCEAN SCIENCES

Algal products

- Health food ($2.5-billion/year)
- Aquaculture ($700-million/year)
- Animal feed additives ($300-million/year)
- PUFAs (DHA) ($1.5-billion/year) (eg Martek)
- Anti-oxidants (eg. Beta-Carotene) ($400-million/yr)
- Coloring substances (eg. Astaxanthin) ($160-million/yr)
- Fertilizers/soil conditioners ($5-billion/yr)



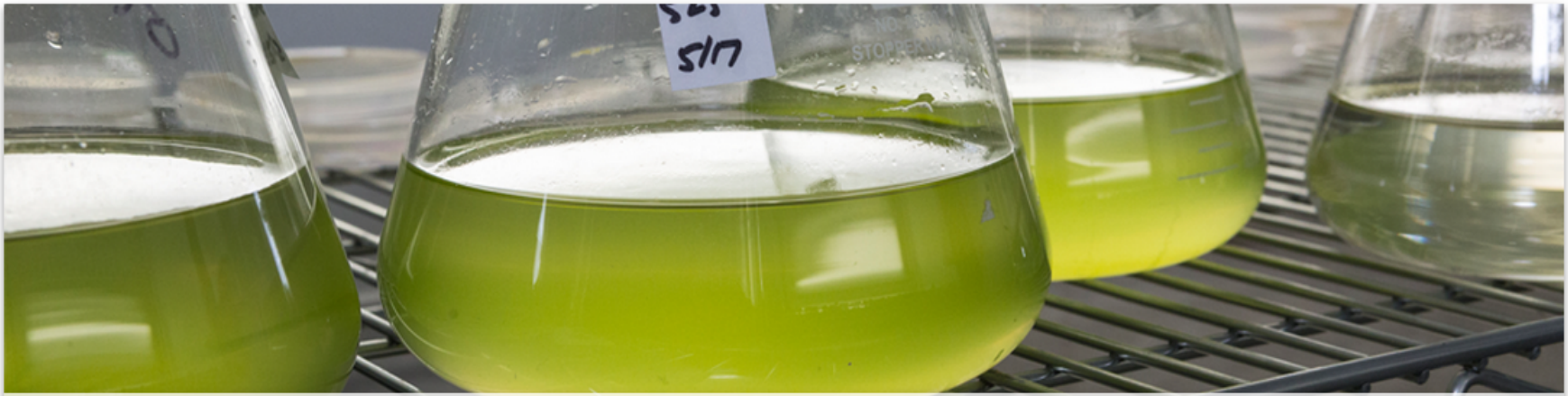
The NCMA holds representatives from 39 Classes of Algae (all the major photosynthetic groups)



Products and Services

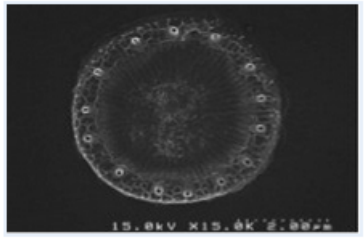
- Starter cultures
- Nucleic acids from algae
- Culturing Techniques Courses
- Private collections
- International Depository Authority (patent depository)
- got algae? T-shirts!
- Research services
- Isolations/clean up/taxonomic ID
- Services through our partners (e.g. powdered algae)
- Other service centers at Bigelow
  - Single Cell Genomics
  - Flow Cytometry
  - Analytical Services
  - Seawater Facility

PROVASOLI-GUILLARD
NCMA
National Center for Marine Algae and Microbiota
Culturing Diversity

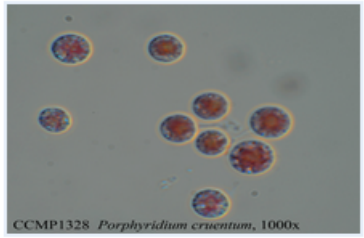


Blue Biotechnology: Harnessing the ocean's potential to benefit society.

Featured Product/Service: Aquaculture Express Strains



Featured Strain: CCMP1335, Thalassiosira pseudonana (aka 3H)



Featured Paper: Genome of the red alga Porphyridium purpureum

Latest News & Announcements

Innovator of the Year Award

Governor Paul LePage presented Bigelow Laboratory for Ocean Sciences with the Maine International Trade Center's (MITC) 2012 Innovator of the Year award

Read More

7th Annual Algae Biomass Summit

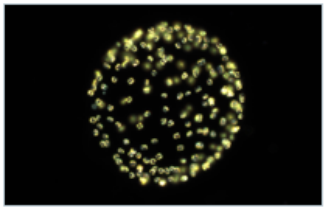
The NCMA is an official sponsor of the 7th annual

Quick Links

Algae Category
Algal Medium Recipes
Tree of Life Search
Search by Location (map)
Quick Order
Make a Deposit

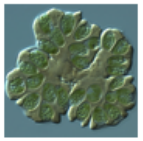
Browse By

Category
All Algae (2715)
Marine (838)
Freshwater (308)
Hypersaline (50)
Heterotrophic and Mixotrophic (0)
Robust (81)
Aquaculture (0)
Toxic (303)
Cold Water (224)
Warm Water (51)
Temperate (72)
High Lipid (0)
Seaweed (125)



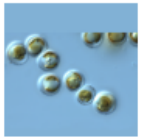
Algae

The NCMA is the largest and most diverse collection of marine algae in the world. With almost half of the cultures in cryopreservation, our curators perpetually culture over 1500 strains. Hundreds of new strains are accessed into the collection each year. The collection is searchable by the Tree of Life, the world map or the following categories.

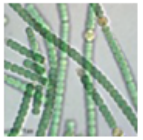


High Lipid
Strains that can be induced to accumulate
View Products

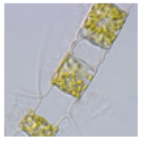
Seaweed
This grouping is based on a generic term
View Products



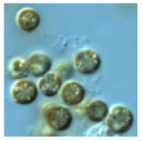
Marine
Strains isolated from the ocean or
View Products



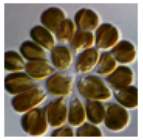
Hypersaline
Strains from an environment with saline
View Products



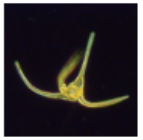
Cold Water
Strains that thrive in temperatures less than
View Products



Warm Water
Strains that are grown at temperatures greater than
View Products



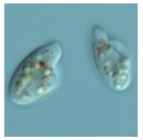
Freshwater
Strains that grow in freshwater media. Thev
View Products



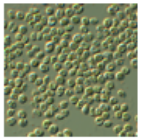
Temperate
Strains that thrive at temperatures between
View Products

Aquaculture
The NCMA aquaculture express starter
View Products

Toxic
Strains that represent species that are known to
View Products

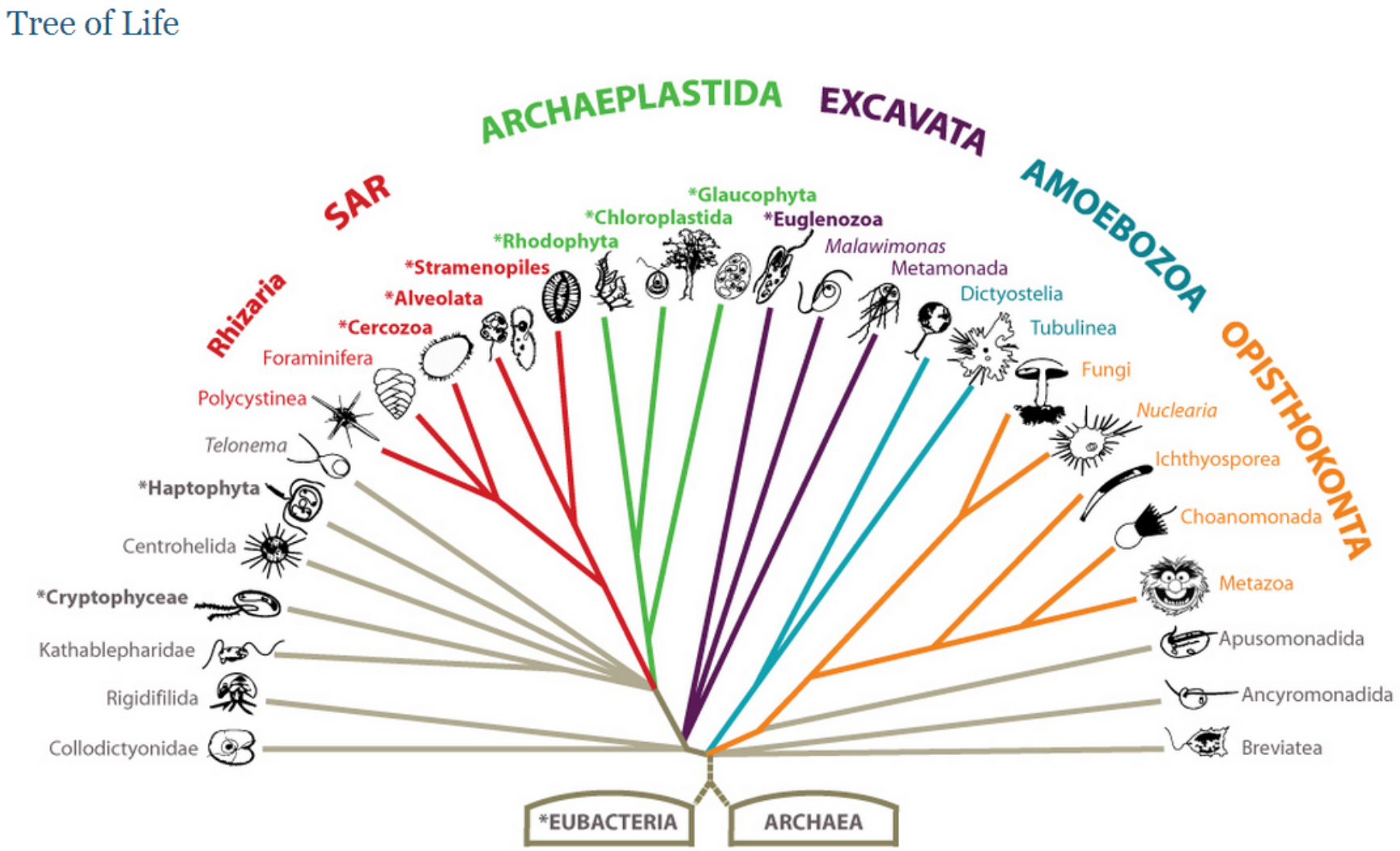


Heterotrophic and Mixotrophic
Heterotrophs require an organic
View Products



Robust
Strains that grow vigorously, do not die
View Products

Interactive Tree of Life



Google Maps

Geographic Map Locator

Enter Location to narrow down your search:

Radius

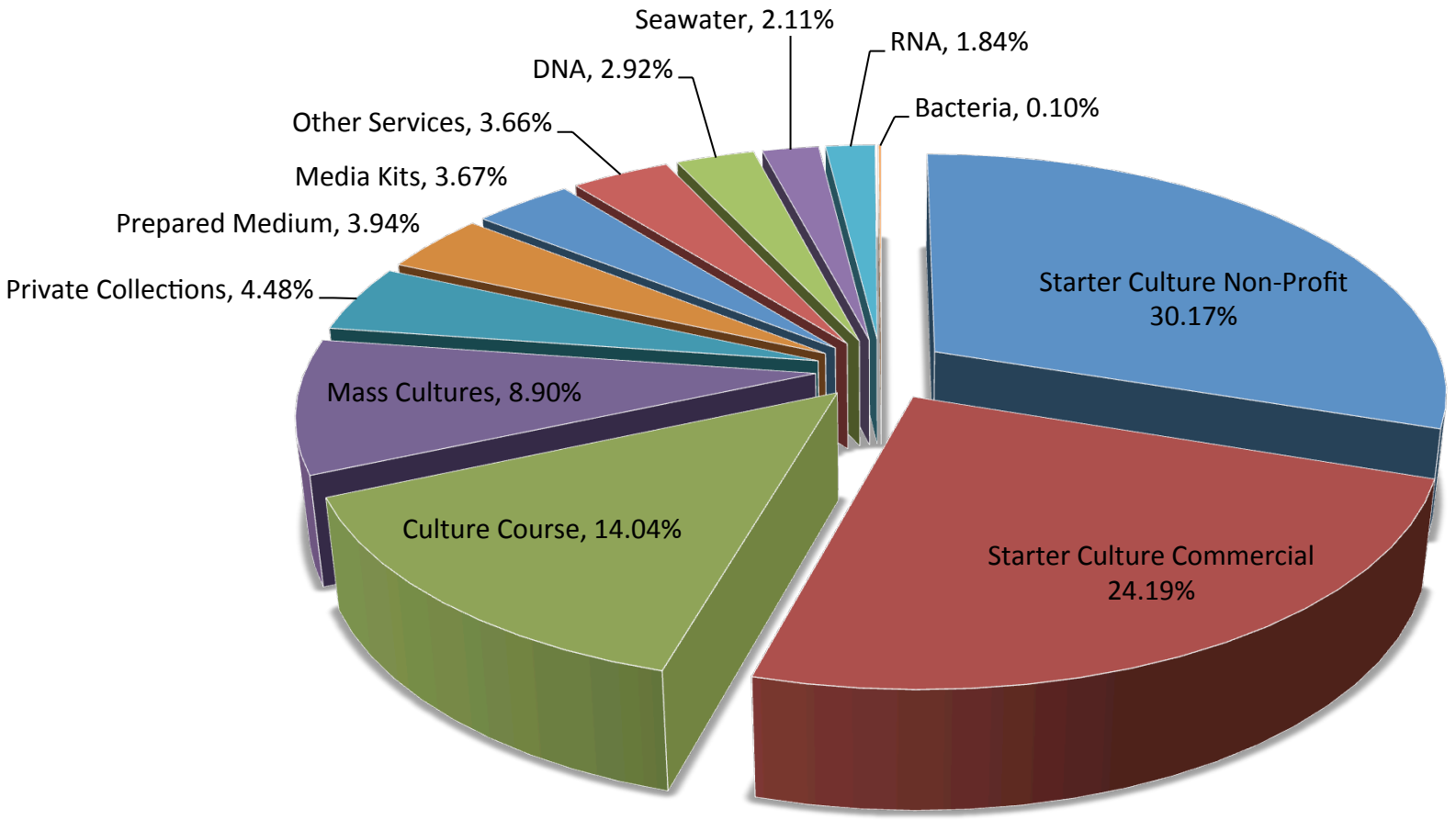
200 mi

Submit Reset

Filter Map by Tag: Show all | Algae Cyanophyceae Prasinophyceae Trebouxiophyceae Fragilariophyceae Coscinodiscophyceae Bacteria **Reset locations**

- CCMP100 - Achnanthes brevipes Falmouth Great Pond; Falmouth; Massachusetts USA
- CCMP1000 - Thalassiosira oceanica continental slope
- CCMP1001 - Thalassiosira oceanica continental slope
- CCMP1002 - Thalassiosira oceanica continental slope
- CCMP1003 - Thalassiosira oceanica Sargasso Sea
- CCMP1004 - Thalassiosira oceanica Gulf Stream Warm Core Ring 81D
- CCMP1005 - Thalassiosira oceanica Sargasso Sea
- CCMP1006 - Thalassiosira

Percent of Gross Revenue by Product FY2009-2012

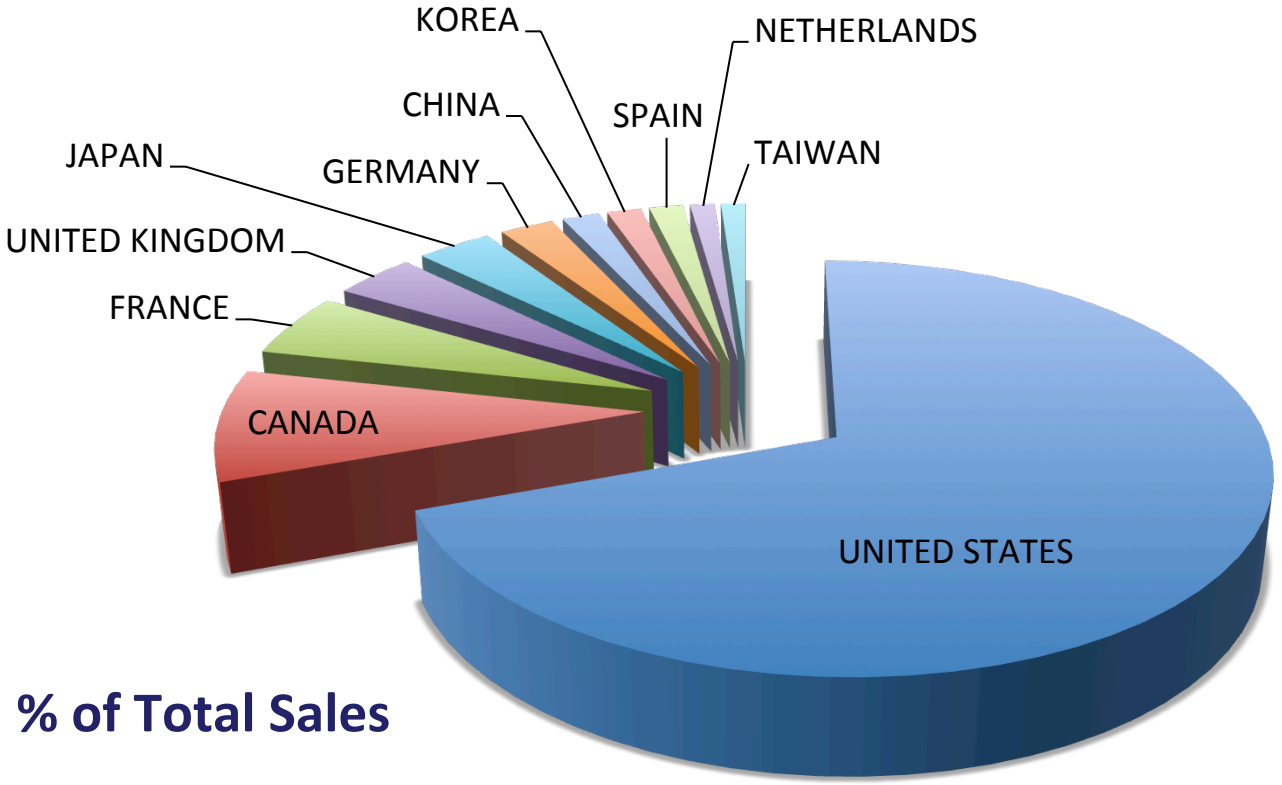


PROVASOLI-GUILLARD NCMA National Center for Marine Algae and Microbiota *Culturing Diversity*

BIGELOW LABORATORY got algae? bigelow.org FOR OCEAN SCIENCES

BIGELOW LABORATORY FOR OCEAN SCIENCES

Percent of Gross Sales by Country (FY2009-2012)



% of Total Sales

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got algae? bigelow.org

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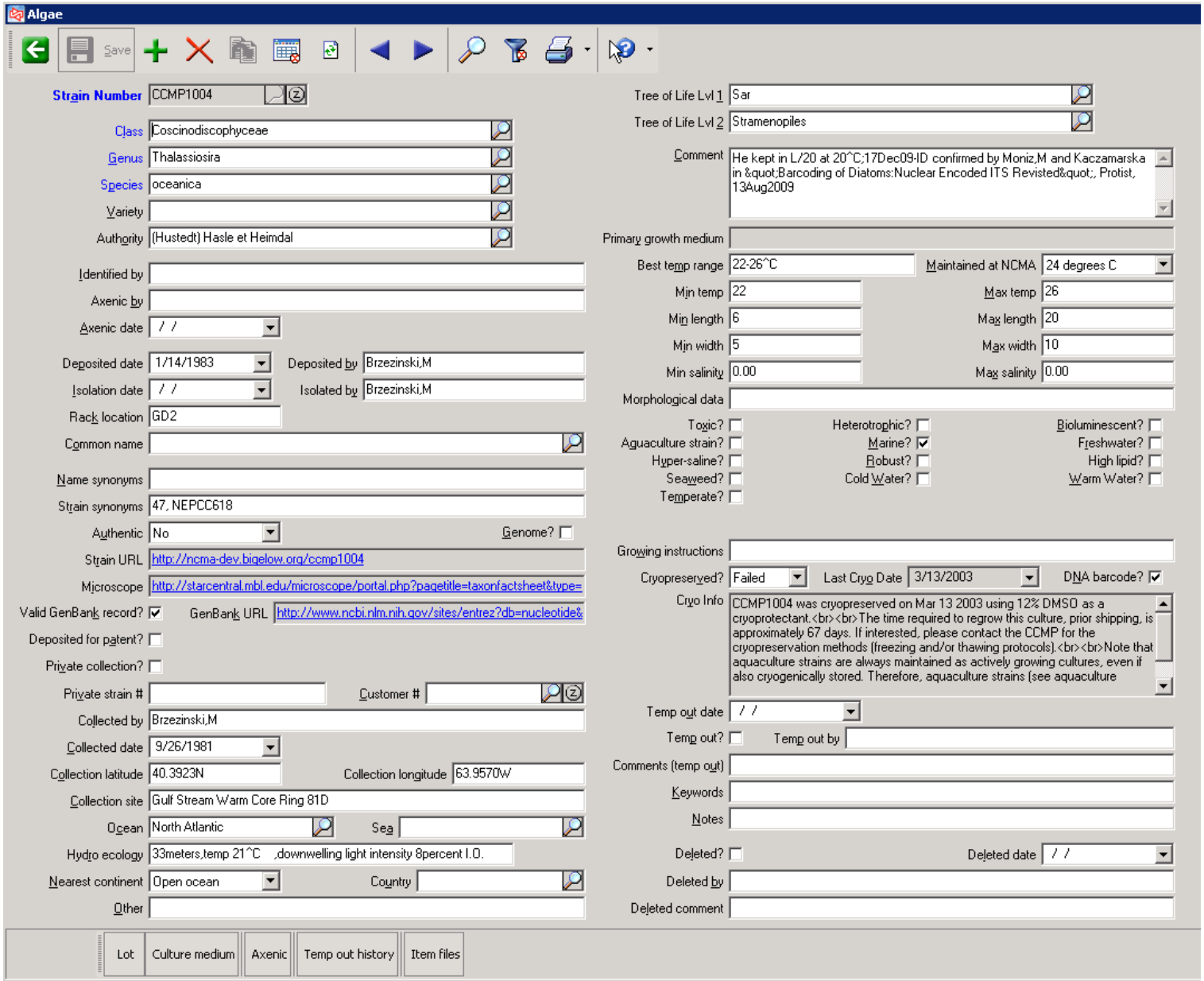
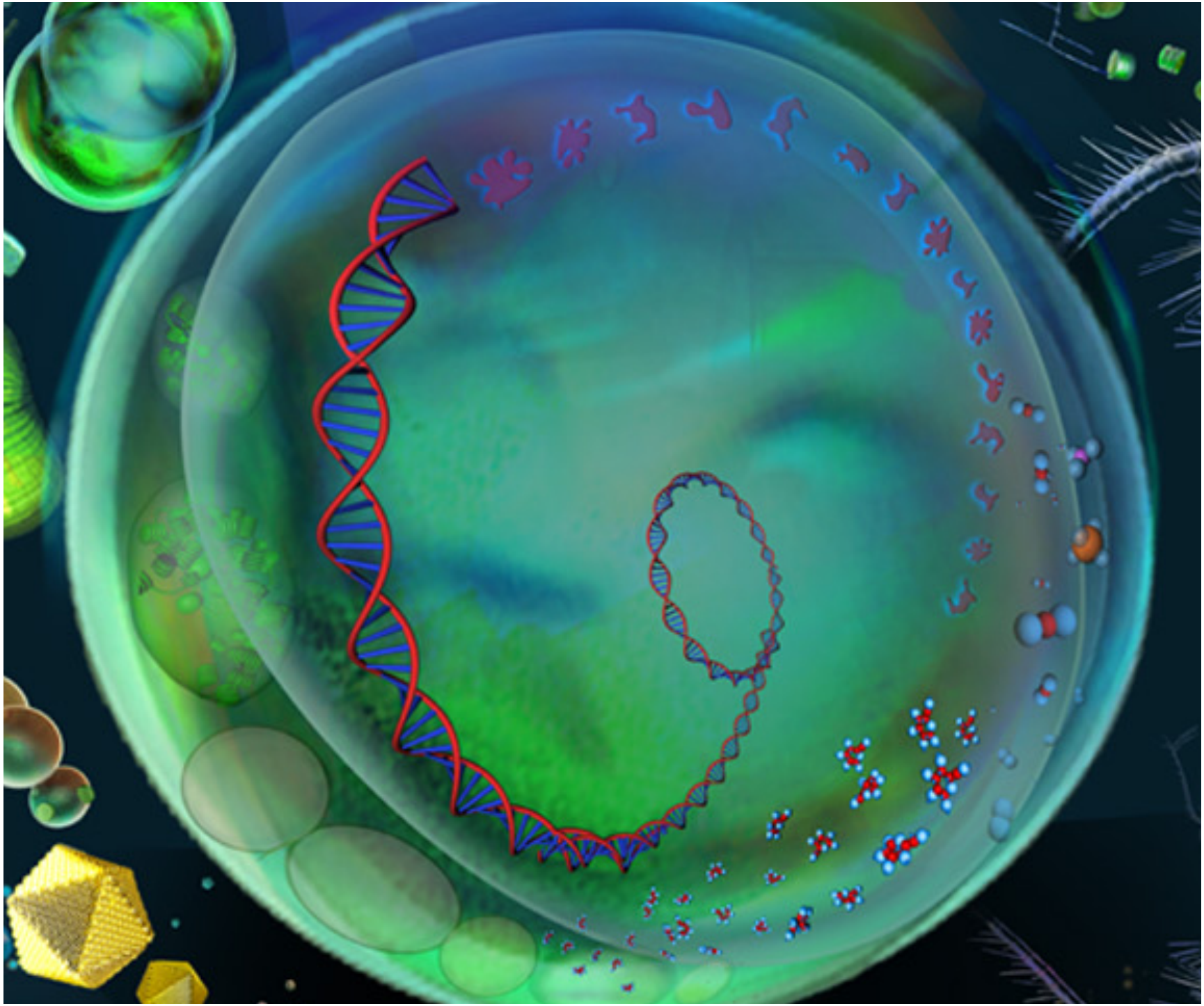
Algae Attributes



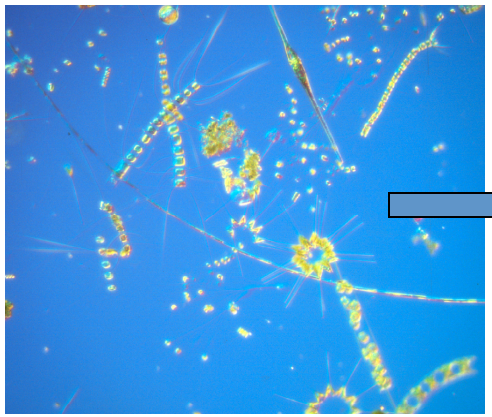


Microbial diversity:

Each mL of ocean water contains $>10^5$ cells

or ~1 terabyte of microbial genetic information!

The metagenomics approach



Extract all the DNA from microbial community

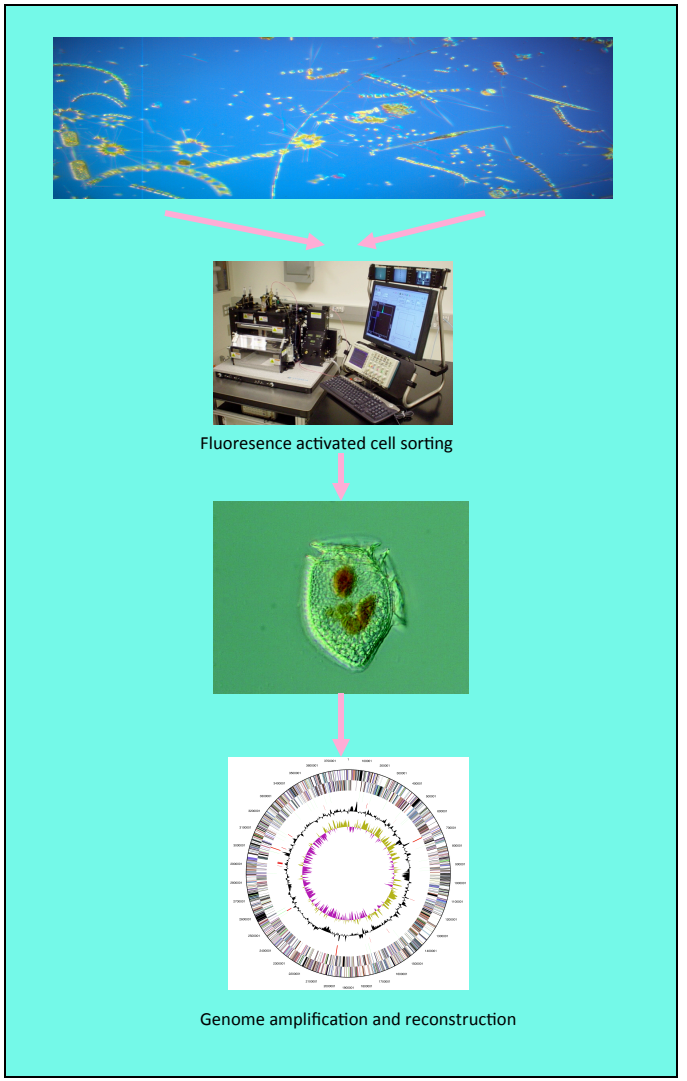
Determine what the genes ARE:
(**Sequence-based metagenomics**)
Identify genes and metabolic pathways
"Who, what and why?"
Requires screening against reference libraries

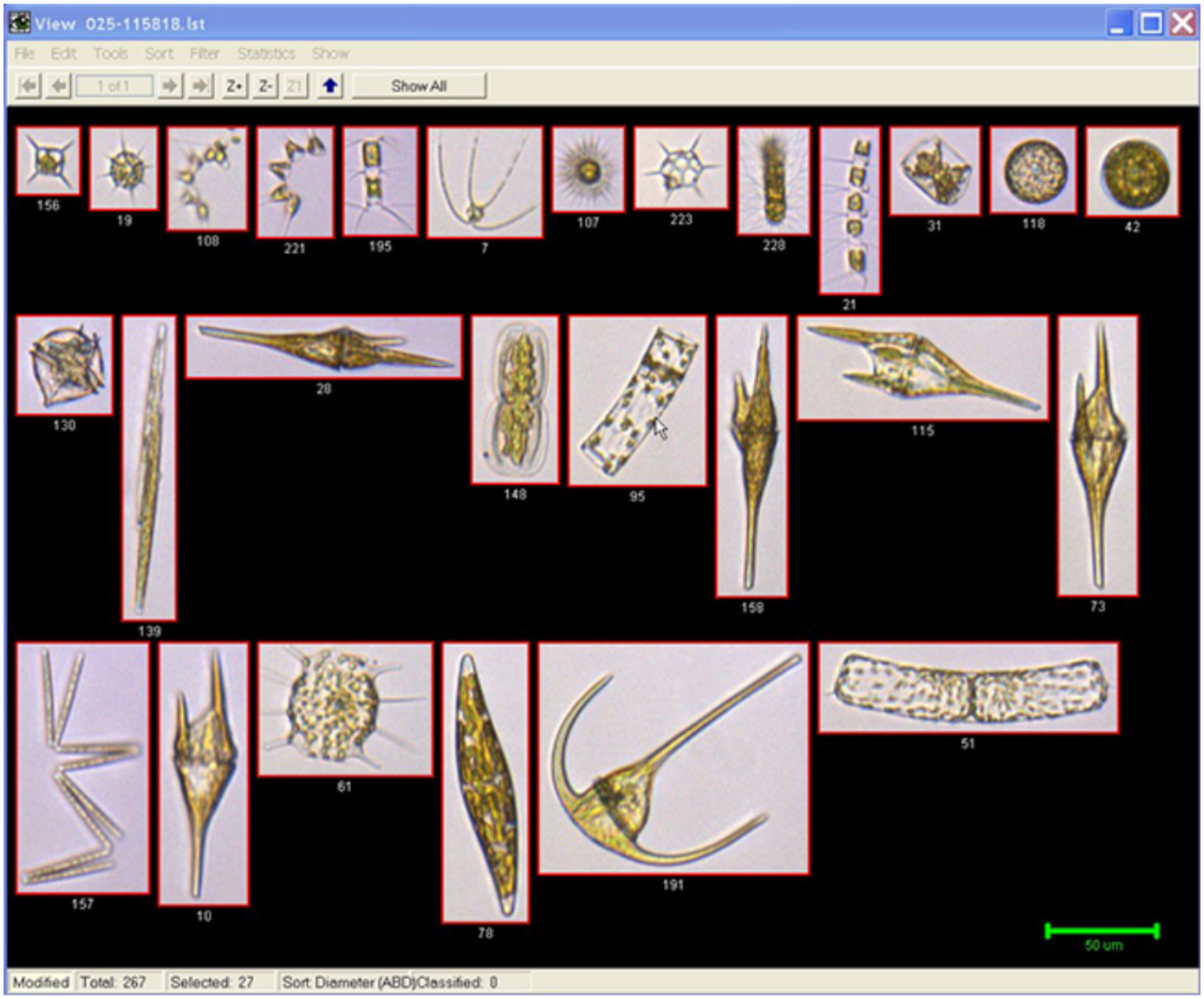
Determine what the genes DO:
(**Function-based metagenomics**)
Screen to identify functions of interest, such as vitamin or antibiotic production
Find the genes that code for functions of interest
Usually, unambiguous assignment of gene function

The National Academies & Kennedy et al, Mar Drugs 2010

Single cell genomics

- Pioneering research led by Bigelow investigators (Stepanauskas, Sieracki et al)
- Applying the power of single cell sorting and genomics together.
- Addresses the limitations of shotgun sequencing and uses vital information on the physiology, environmental factors and evolution to address microbial diversity in the ocean.





1

Nice Harbor, 10X

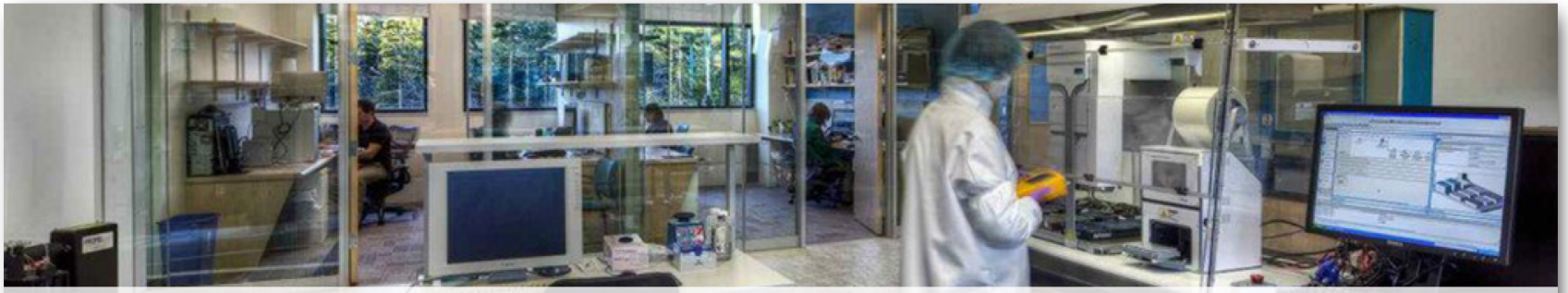
Nice, France

Particle Sorting with Flow Cytometry

BIGELOW LABORATORY SINGLE CELL GENOMICS CENTER

Reading nature's genetic tales, one cell at a time

Products & Services | News & Publications | Education | Resources | About



High-throughput single cell separation, DNA amplification, sequencing and bioinformatics

Announcements & Events:

UPCOMING CELL SORTING SESSIONS:
- November 19th - 22nd, 2013
- January 21st - 24th, 2014

Our new website is unveiled, please **tell us** what you think!

Recent instrumentation awards by **Illumina** and **NSF** will boost SCGC's genomic sequencing capacity

Bigelow Laboratory receives **State of Maine Innovator of the Year** and top **AGC Build New England** awards

Single cell genomics technology and applications are poised for **rapid growth**

Recent SCGC-enabled discoveries:

Biology's Dark Matter Illuminated

A collaborative study led by the U.S. Department of Energy Joint Genome Institute sheds light on metabolic features, evolutionary histories and global distribution of 28 major branches of Bacteria and Archaea that have been largely unknown to date, due to their evasion from laboratory cultivation.

*Original publication: **Rinke et al. 2013; Nature 499:431-437***

Resourceful Microbes Reign in World's Oceans

In the first large-scale single cell genomics project, a research team led by SCGC scientists discovered that marine microbes are adapted to very narrow and specialized niches in their environment. This may explain why so few of these microbes—usually less than 1%—can be grown for study in the laboratory.

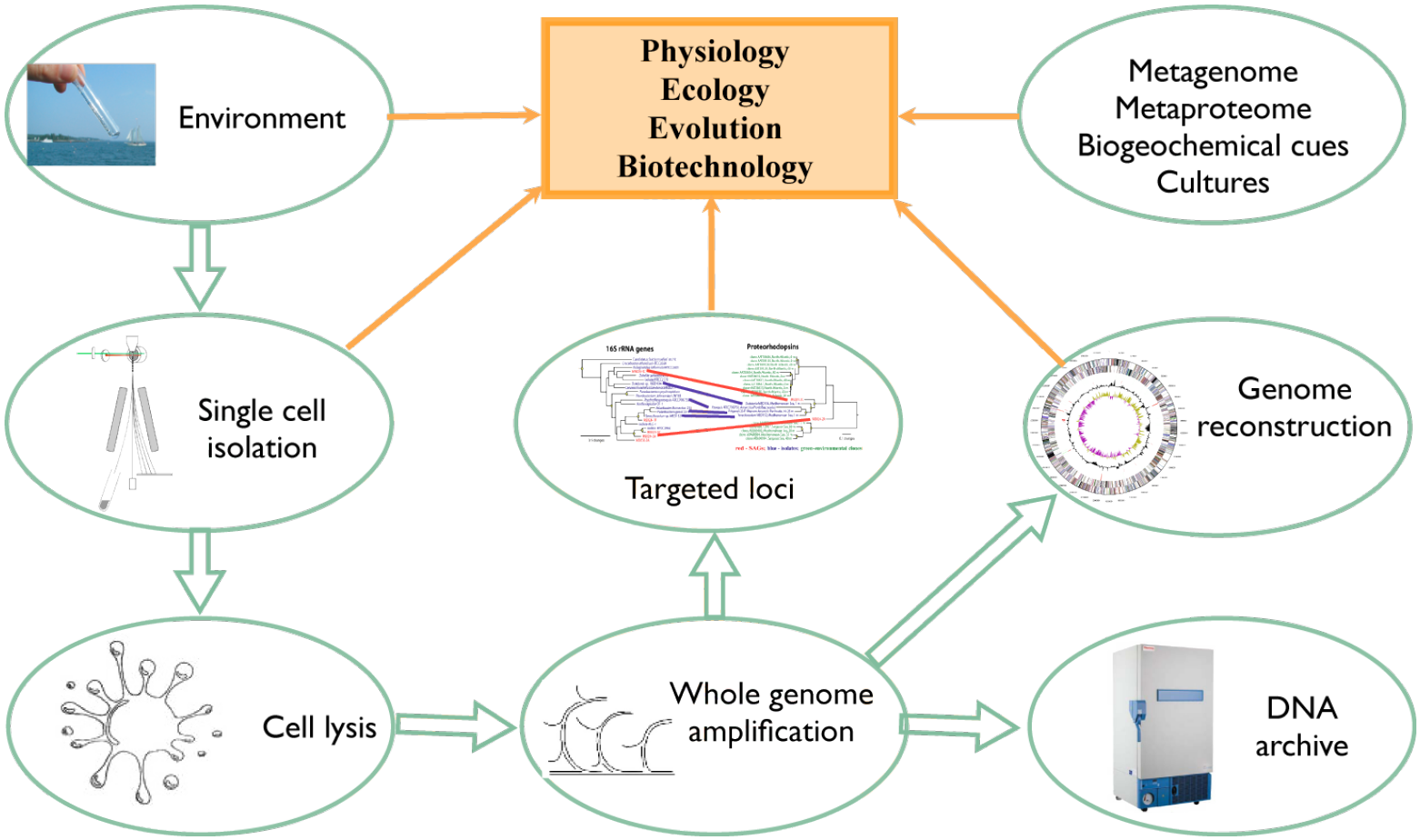
*Original publication: **Swan et al. 2013; PNAS 110:11463-11468***

Intraterrestrial Lifestyles of Marine Archaea Revealed

A study led by scientists from the Aarhus University and the University of Tennessee reveals metabolic features of two lineages of Archaea that are ubiquitous in marine sediments worldwide, suggesting that they may play a key role in the global degradation of buried protein.

*Original publication: **Lloyd et al. 2013; Nature 496:215-218***

Single Cell Genomics Pipeline

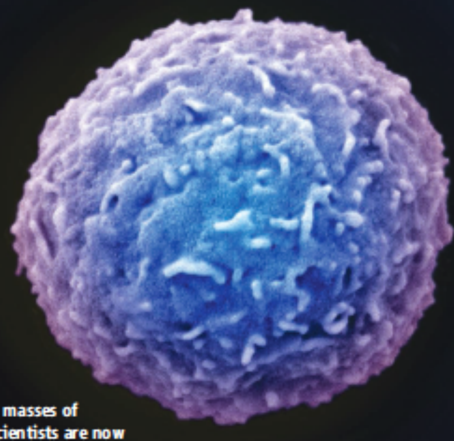


Single Cell Genomics: Dr Ramunas Stepanuskas

CELL BIOLOGY

The Power Of One

Rather than probe masses of cells all at once, scientists are now applying new research techniques to individual cells



Small sample size. Researchers are exploring new ways of investigating individual cells such as this human white blood cell.

1) Measure out a heaping portion of cells.
2) Grind until thoroughly mixed.
3) Analyze.

Cellular studies still pretty much stick to this traditional recipe, whether the goal is probing bacterial metabolism, following differentiation of stem cells, or tabulating gene activity in tumors. But mashing up a multitude of cells—one common method of studying gene expression typically requires more than 10,000—obliterates key differences between cells, researchers have come to realize. "If you take an average of a large number of cells, you get an average answer," says analytical chemist Renato Zenobi of the Swiss Federal Institute of Technology in Zurich.

That's why more and more scientists are opting for the alternative approach of taking the measure of individual cells. Although much of this work is in its early stages, "there is an increasingly diverse set of examples where single-cell studies have provided qualitative insights that couldn't be obtained from population-level studies," says biophysicist Michael Elowitz of the California Institute of Technology in Pasadena.

Scientists have already recorded the most accurate measurements of how much an individual cell weighs and gauged how much oxygen one requires. They've flagged specific cancer cells resistant to chemotherapy and developed ways to pinpoint rare, disease-causing bacteria among swarms of harmless microbes. Developmental biologists have tallied gene activity as a fertilized egg starts its course of division and specialization, work that might help clarify the factors that spur a cell in the embryo to become one tissue and its seemingly identical next-door neighbor to become something else. And Elowitz and other researchers have spelled out how individual cells not only cope with but actually benefit from "noise," random fluctuations in their internal and external conditions.

Of course, scientists have paid attention to single cells ever since the first microscopes were invented. What's changed is that researchers are now applying to individual cells the powerful techniques, including genome sequencing, mass spectrometry, and gene expression analysis, that formerly required batches of cells. "Real biological tissues are complex, and if you want to dissect that complexity and heterogeneity, you have to have tools to do it at the single-cell level," says biophysicist Stephen Quake of Stanford University in Palo Alto, California.

Good technique

Single-cell research tools range from old standbys to cutting-edge inventions (see sidebar for a sample of methods). Many of them allow researchers to get into what Quake calls "production mode," analyzing large numbers of individual cells in parallel or over a short period of time. The technology he calls "absolutely central" to the surge in single-cell research is microfluidics, which uses miniaturized networks of channels, valves, pumps, and chambers to control microscopic quantities of liquid. So-called lab-on-a-chip devices combine microfluidic circuits and can perform several analytical steps.

An example of how microfluidics can elucidate single-cell behavior comes from Quake, his Stanford colleague Markus Covert, and their colleagues. Last July in *Nature*, the

Single-Cell Tech Primer

Microfluidics is the hot technique in the single-cell field (see main text). However, it's just one of the methods that are enabling researchers to delve into individual cells.

• Gene Expression

Many modern gene-expression studies apply the mingled contents of thousands of cells to devices called microarrays that look like glass slides or microchips. But with microfluidic chips, researchers can make the same measurements on one cell. Take the gene-expression chips from California-based Fluidigm, a company co-founded by Stephen Quake of Stanford University in Palo Alto, California. The devices isolate samples from individual cells and mix them with the chemicals necessary for quantitative polymerase chain reaction, a technique that determines gene activity by measuring how much messenger RNA a gene makes. The company's most powerful version allows researchers to simultaneously gauge the activity of 96 genes in 96 individual cells, churning through a batch in about 4 hours.

Another new technology, known as RNA-seq, provides an alternative to microfluidic chips for measuring gene activity in one cell. An offshoot of next-generation genome sequencing, the procedure involves converting a cell's mRNA molecules back into short strands of DNA, sequencing those DNA fragments, and then matching them up with the gene that originally spawned the mRNAs. Last May in *Cell Stem Cell*, molecular geneticist M. Azim Surani of the University of Cambridge in the United Kingdom and colleagues reported one of the first studies to apply the RNA-seq technique to single cells, revealing the expression of 385 genes in individual embryonic stem cells. Fans of RNA-seq emphasize that it can measure more genes at once than can microfluidic chips. Meanwhile, chip devotees tout their method's superior speed.

• Flow Cytometry

A classic technique, flow cytometry sifts and counts cells based

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Dark ocean autotrophy

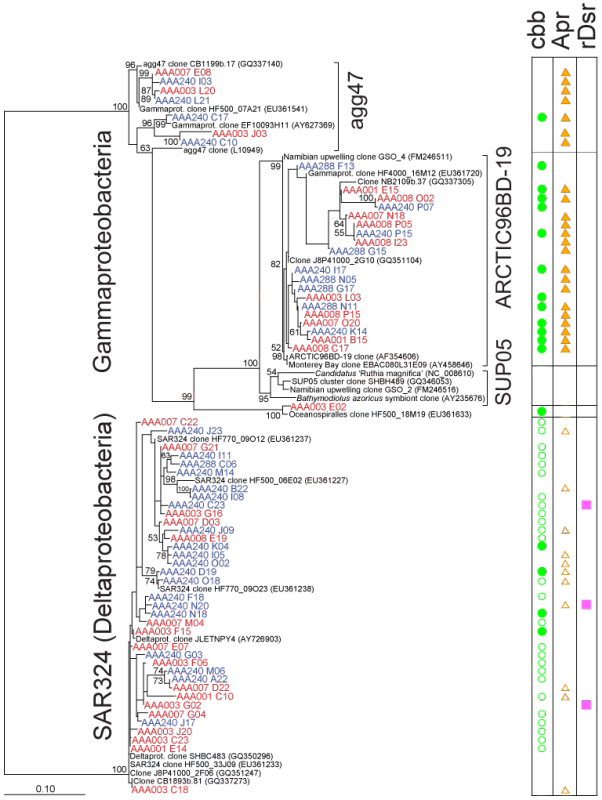
Lead postdoc:
Brandon Swan

RuBisCO and S oxidation genes detected in SAR324 and γ-Proteobacteria

Consistent in multiple cells from S. Atlantic and N, Pacific

May be significant players in the global carbon cycle

16S rDNA phylogeny and the presence of metabolic genes

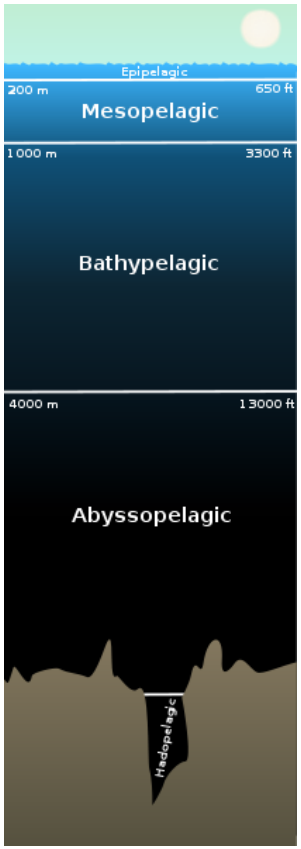


South Atlantic
North Pacific

Rubisco (cbb)
- Form II
- Form IA

APS reductase (Apr)
- SOB lineage I
- SOB lineage II

- Reverse sulfite reductase (rDsr)



Science, **333**, 2011

Ecology of Picobiliphyta

Newly discovered marine protist group

No cultures

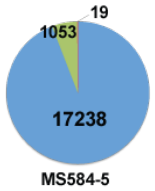
Our findings:

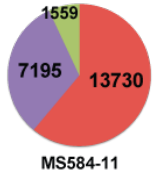
- No photosynthesis

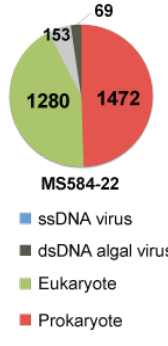
- Feed on bacteria and large viruses

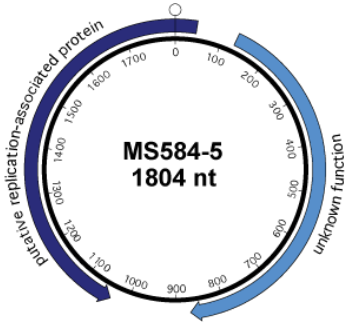
- infected by a novel nanovirus

Shotgun read sources



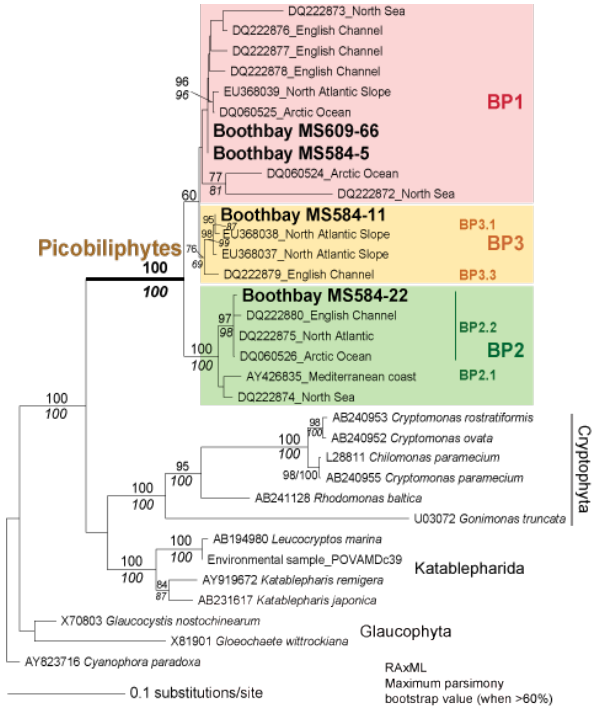




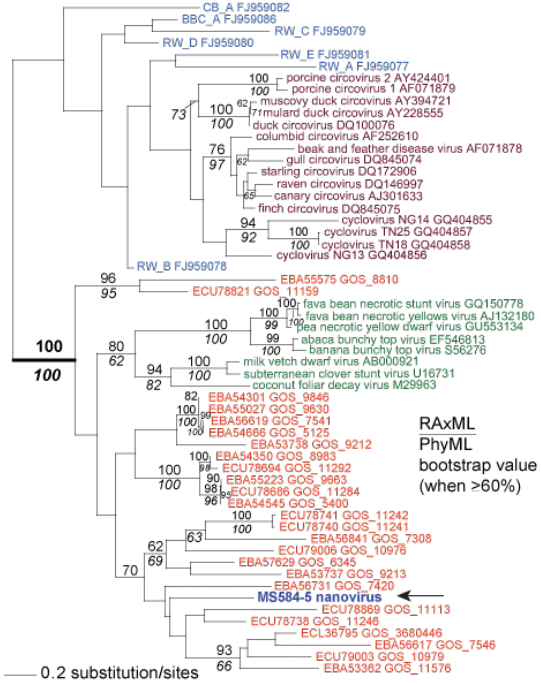


Lead PIs:
HS Yoon (Bigelow)
D Bhattacharya (Rutgers U)

18S rDNA phylogeny



Picobiliphyta-infecting nanovirus



Science, **332,** 2011

Genomic Encyclopedia of Bacteria and Archaea (GEBA)

led by T Woyke (DOE JGI)

Goals:

- Representing each major branch of the tree of life with a genome
- Discovering of genes, proteins and pathways
- Resolving early evolution of life

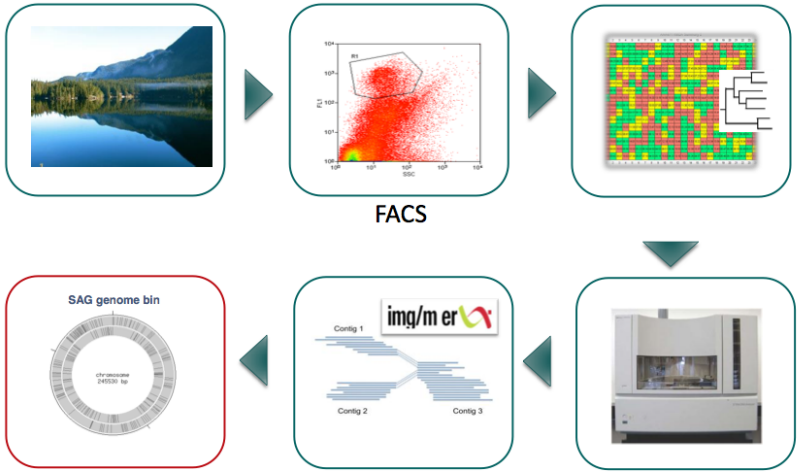
Methods:

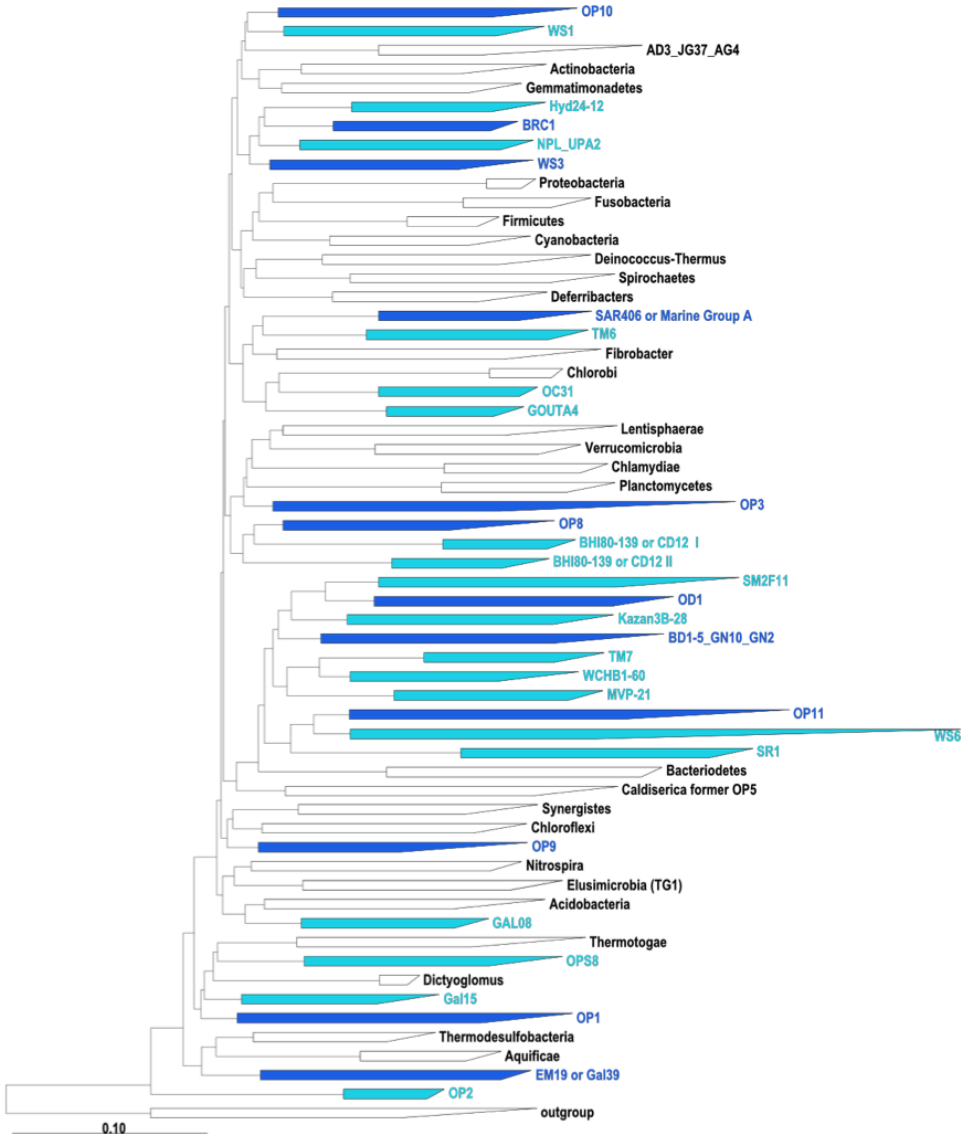
-Single cell FACS and MDA (Bigelow SCGC)

-Whole genome sequencing (JGI)

Uncultured groups represented so far:

- ~15 Bacteria phyla
- ~10 Archaea phyla





Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean

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Edited by W. Ford Doolittle, Dalhousie University, Halifax, Canada, and approved May 28, 2013 (received for review March 7, 2013)

Planktonic bacteria dominate surface ocean biomass and influence global biogeochemical processes, but remain poorly characterized owing to difficulties in cultivation. Using large-scale single cell genomics, we obtained insight into the genome content and biogeography of many bacterial lineages inhabiting the surface ocean. We found that, compared with existing cultures, natural bacterioplankton have smaller genomes, fewer gene duplications, and are depleted in guanine and cytosine, noncoding nucleotides, and genes encoding transcription, signal transduction, and noncytoplasmic proteins. These findings provide strong evidence that genome streamlining and oligotrophy are prevalent features among diverse, free-living bacterioplankton, whereas existing laboratory cultures consist primarily of copiotrophs. The apparent ubiquity of metabolic specialization and mixotrophy, as predicted from single cell genomes, also may contribute to the difficulty in bacterioplankton cultivation. Using metagenome fragment recruitment against single cell genomes, we show that the global distribution of surface ocean bacterioplankton correlates with temperature and latitude and is not limited by dispersal at the time scales required for nucleotide substitution to exceed the current operational definition of bacterial species. Single cell genomes with highly similar small subunit rRNA gene sequences exhibited significant genomic and biogeographic variability, highlighting challenges in the interpretation of individual gene surveys and metagenome assemblies in environmental microbiology. Our study demonstrates the utility of single cell genomics for gaining an improved understanding of the composition and dynamics of natural microbial assemblages.

comparative genomics | marine microbiology | microbial ecology | microbial microevolution | operational taxonomic unit

Planktonic bacteria dominate surface ocean biomass and have a major impact on the global cycling of carbon, nitrogen, and other elements (1). Among the available pure cultures of marine bacterioplankton, only a limited number represent bacterioplankton that are abundant in the ocean, such as the cyanobacteria *Prochlorococcus* and *Synechococcus* and the Alphaproteobacteria *Pelagibacter* (collectively termed PSP cultures). This limits the scope of studies of the microbial metabolic processes and evolutionary changes that impact marine ecosystems and their geochemical cycles (2–6). Unusual nutritional requirements resulting from genome reduction may contribute to cultivation difficulties, as suggested by studies of the chemoheterotroph *Pelagibacter* (7, 8) and the methylotroph OM43 (9).

Although prevailing culture-independent tools, including microbial community shotgun sequencing, targeted gene surveys, and fluorescent in situ hybridization, have revealed the extent and significance of microbial diversity, they have not been able to provide the genome context information required for accurate metabolic reconstruction spanning organismal, population, and community levels of organization (10). As a result, the genomic repertoires, natural histories, and geographic distribution of even the most abundant taxonomic groups of marine bacterioplankton remain largely unknown (1, 11). Microbial studies in other environments, such as the human body and soils, face similar challenges (10). The recent development of robust protocols for single cell genomics provides a versatile, cultivation-independent approach for assessing natural microbial diversity with corresponding genome context information (12).

To determine whether genome streamlining is a prevalent feature among free-living marine bacterioplankton, and to analyze global patterns of surface ocean bacterioplankton distribution, we obtained draft genomes of 56 single amplified genomes (SAGs) (5, 13–15) and compared them with existing bacterioplankton cultures and metagenomes. The sequenced SAGs represent many ubiquitous surface ocean bacteria lineages, including Marine Group A, Verrucomicrobia, Actinobacteria, Bacteroidetes, and Proteobacteria lineages SAR86, ARCTIC96BD-19, SAR92, SAR116, and Roseobacter (*SI Appendix*, Fig. S1). The majority of these groups have few or no cultured representatives. Members of the PSP group were excluded from SAG selection, because their genome streamlining and environmental abundance have been demonstrated previously (1, 2, 4, 11). Samples for SAG generation were collected from the Gulf of Maine, the Mediterranean Sea, and the subtropical gyres of the North Pacific and South Atlantic Oceans (*SI Appendix*, Table S1). On average, 55% (range, 0.3–97.8%) of the genome was recovered from each analyzed cell (*SI Appendix*, Table S2). A subset of 41 SAGs, each >0.75 Mbp in size and with >30% estimated genome recovery, was used for our

Author contributions: B.K.S., B.T., A.S., and R.S. designed research; B.K.S., B.T., A.S., F.M.L., M.M.-G., J.M.G., H.L., J.J.W., Z.C.L., N.W.H., B.P.T., N.J.P., S.G.A., T.W., and R.S. performed research; B.K.S., B.T., A.S., T.W., and R.S. contributed new reagents/analytic tools; B.K.S., B.T., A.S., F.M.L., M.M.-G., J.M.G., H.L., J.J.W., Z.C.L., N.W.H., P.S., S.G.A., S.J.G., M.A.M., S.J.H., R.C., T.W., and R.S. analyzed data; and B.K.S. and R.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: Whole-genome sequence data for single amplified genomes used for our analyses are available in the Joint Genome Institute's Integrated Microbial Genome database, http://img.jgi.doe.gov/cgi-bin/w/main.cgi (accession nos. 643386079, 643886118, 2228664025–26, 2228664028–29, 2228664032, 2228664034, 2228664053–53, 2228664055–56, 2236347001, 2236347003, 2236347013, 2236347015, 2236347017–19, 2236347021–24, 2236347026–27, 2236347030–33, 2236347035–36, 2236347039, 2236347041, 2236347043, 2236661010, 2236661014, 2236661017–18, 2507262045, 2507262047, and 2517572139).

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ENVIRONMENTAL SCIENCES

ARTICLE

OPEN

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Insights into the phylogeny and coding potential of microbial dark matter

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Genome sequencing enhances our understanding of the biological world by providing blueprints for the evolutionary and functional diversity that shapes the biosphere. However, microbial genomes that are currently available are of limited phylogenetic breadth, owing to our historical inability to cultivate most microorganisms in the laboratory. We apply single-cell genomics to target and sequence 201 uncultivated archaeal and bacterial cells from nine diverse habitats belonging to 29 major mostly uncharted branches of the tree of life, so-called 'microbial dark matter'. With this additional genomic information, we are able to resolve many intra- and inter-phylum-level relationships and to propose two new superphyla. We uncover unexpected metabolic features that extend our understanding of biology and challenge established boundaries between the three domains of life. These include a novel amino acid use for the opal stop codon, an archaeal-type purine synthesis in Bacteria and complete sigma factors in Archaea similar to those in Bacteria. The single-cell genomes also served to phylogenetically anchor up to 20% of metagenomic reads in some habitats, facilitating organism-level interpretation of ecosystem function. This study greatly expands the genomic representation of the tree of life and provides a systematic step towards a better understanding of biological evolution on our planet.

Microorganisms are the most diverse and abundant cellular life forms on Earth, occupying every possible metabolic niche. The large majority of these organisms have not been obtained in pure culture and we have only recently become aware of their presence mainly through cultivation-independent molecular surveys based on conserved marker genes (chiefly small subunit ribosomal RNA; SSU rRNA) or through shotgun sequencing (metagenomics)[1,2]. As an increasing number of environments are deeply sequenced using next-generation technologies, diversity estimates for Bacteria and Archaea continue to rise, with the number of microbial 'species' predicted to reach well into the millions[3]. According to SSU rRNA-based phylogeny, these fall into at least 60 major lines of descent (phyla or divisions) within the bacterial and archaeal domains[4], of which half have no cultivated representatives (so-called 'candidate' phyla). This biased representation is even more fundamentally skewed when considering that more than 88% of all microbial isolates belong to only four bacterial phyla, the Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes (Supplementary Fig. 1a). Genome sequencing of microbial isolates naturally reflects this cultivation bias (Supplementary Fig. 1b). Recently, a systematic effort, the Genomic Encyclopaedia of Bacteria and Archaea (GEBA) Project[5], has been initiated to maximize coverage of the diversity captured in microbial isolates by phylogenetically targeted genome sequencing. However, GEBA does not address candidate phyla that represent a major unexplored portion of microbial diversity, and have been referred to as microbial dark matter (MDM)[6].

Metagenomics can obtain genome sequences from uncultivated microorganisms through direct sequencing of DNA from the environment[7]. In some instances, draft or even complete genomes of candidate phyla have been recovered solely from metagenomic data (Supplementary Table 1). A complementary cultivation-independent approach for obtaining genomes from candidate phyla is single-cell genomics; the amplification and sequencing of DNA from single cells obtained directly from environmental samples[8]. This approach can be used for targeted recovery of genomes and has been applied to members of several candidate phyla (Supplementary Table 1). In particular, natural populations that have a high degree of genomic heterogeneity will be more accessible through single-cell genomics than through metagenomics as co-assembly of multiple strains is avoided. Despite these advances in obtaining genomic representation of MDM, no systematic effort has been made to obtain genomes from uncultivated candidate phyla using single-cell whole genome amplification approaches.

Here, we present GEBA-MDM, the natural extension of the Genomic Encyclopaedia into uncultivated diversity by applying single-cell genomics to recover draft genomes from over 200 cells representing more than 20 major uncultivated archaeal and bacterial lineages. Genome-based phylogenetic analysis confirms the validity of rRNA-defined candidate phyla as monophyletic groups and resolves a number of associations among phyla not apparent by single gene analysis. We discovered several unexpected features, including archaeal sigma factors and stop codon reassignments that challenge established views of the microbial world. Furthermore, we show that single-cell genome references substantially improve the phylogenetic anchoring of about 340 million previously incorrectly or under-classified metagenomic reads.

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Antimicrobials • Genomics

Single cell genomics: an individual look at microbes

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Open Access

Single cell genomics (SCG) uncovers hereditary information at the most basic level of biological organization. It is emerging as a powerful complement to cultivation-based and microbial community-focused research approaches. SCG has been instrumental in identifying metabolic features, evolutionary histories and inter-organismal interactions of the uncultured microbial groups that dominate many environments and biogeochemical cycles. The SCG approach also holds great promise in microbial microevolution studies and industrial bioprospecting. Methods for SCG consist of a series of integrated processes, beginning with the collection and preservation of environmental samples, followed by physical separation, lysis and whole genome amplification of individual cells, and culminating in genomic sequencing and the inference of encoded biological features.

Highlights

► Single cell genomics (SCG) analyzes DNA at the most fundamental level of biology. ► SCG uncovers metabolic potential of the uncultured microorganisms. ► *In situ* studies of microbial predation, infections and symbioses are enabled. ► SCG will improve our understanding of prokaryote diversity and diversification. ► Genomic bioprospecting of the microbial uncultured majority is enabled by SCG.

Press Releases

Publications

PRESS RELEASES AND MEDIA COVERAGE OF SCGC ACTIVITIES

2013

e! Science News [Boldly illuminating biology's 'dark matter'. e! Science News, July 2013]

Bigelow Laboratory [Bigelow Laboratory Single Cell Genomics — Working to Illuminate Biology's "Dark Matter". Bigelow Laboratory for Ocean Sciences, July 2013]

Bigelow Laboratory [Bigelow Scientists Explore Deep Biosphere Under North Pacific Ocean. Bigelow Laboratory for Ocean Sciences, July 2013]

PBS NEWSHOUR [Jacobson R. New Organisms Shine a Light on 'Microbial Dark Matter'. PBS, July 2013]

TheScientist [Yong E. Microbial Diversity. The Scientist, July 2013]

genomeweb [Hundreds of Microbial Genomes Sequenced by Single-Cell Genomics. GenomeWeb, July 2013]

nature REVIEWS MICROBIOLOGY [Hofer U. Exploring Diversity with Single Cell Genomics, July 2013]

Science [Pennisi E. How Many Microbes Are Hiding Among Us? Science, July 2013]

ScienceDaily [Resourceful Microbes Reign in World's Oceans. ScienceDaily, July 2013]

SCIENTIFIC AMERICAN™ [Hayden EC (2013) Single-Cell Sequencing Reveals Genomes of More Than 200 Unusual Microbes. Scientific American, July 2013]

Service Description

Inquiry/Request

Preparation & Shipment

Corporate Partnerships

Terms & Conditions

SERVICE DESCRIPTION

Overview

Single cell genomics consists of a series of integrated processes, starting with appropriate collection and preservation of environmental samples, followed by physical separation, lysis, and whole genome amplification of individual cells, then proceeding to either targeted loci or whole genome sequencing and sequence interpretation. SCGC offers a comprehensive suite of single cell genomics services, from single cell separation through genome sequencing and bioinformatics. SCGC also provides advice on environmental sample collection and storage protocols (please see shipping instructions) and post-sequencing analyses (customized services). Most general methods that are currently employed by SCGC have been described previously (e.g. Swan et al. 2011). However, please keep in mind that continued method improvement is a significant compotent of SCGC activities, and many details of our protocols keep evolving.

A detailed description of SCGC services is available for **download as a PDF file**.

Click on individual services in the table below for descriptions. References are available for download.

Service	Cat. #	Unit	Bigelow	Other Nonprofit	Corporate
Single Amplified Genome (SAG) Generation	S-101	384-well plate	$2,700	$3,200	$5,400
Bacteria SAG identification	S-102	384-well plate	$2,200	$2,600	$4,400
Archaea SAG identification	S-103	384-well plate	$2,200	$2,600	$4,400
Eukarya SAG identification	S-104	384-well plate	$2,200	$2,600	$4,400
SAG re-arraying	S-105	96-well plate	$400	$450	$800
Prokaryote SAG Whole Genome Sequencing	S-014	1 SAG	$3,000	$3,600	$6,000
SAG re-MDA	S-007	1 SAG	$400	$450	$800
Consultation	S-011	1 hour	free	$260	$450
Customized Services	S-100		Req. a quote	Req. a quote	Req. a quote

Single Cell Genomics Center
Bigelow Laboratory for Ocean Sciences
Tel: 207-315-2567, ext. 517
Dr. Ramunas Stepanauskas, *SCGC Director:* rstepanauskas@bigelow.org

Human gut flora (bacteria)

- 100 trillion viable bacteria
- 10x total # cells in the body
- Comprise about 2lbs in mass
- Role of many bacteria is not well understood
- Single cell genomics may offer a new and powerful approach

sp11symbiosis.providence.wikispaces.net

IUCN Information Papers

for the

Intersessional Workshop on Marine Genetic Resources 2-3 May 2013

United Nations General Assembly Ad Hoc Open-ended Informal Working Group to study issues relating to the conservation and sustainable use of marine biological diversity beyond areas of national jurisdiction

Prepared by
IUCN Environmental Law Centre

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