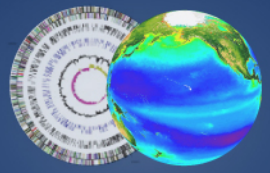
Pelagibacter - the most abundant heterotroph in the oceans

Michael S. Rappé

June 7, 2010
Agouron Summer Course



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~~*Pelagibacter*~~ The SAR11 clade - the most abundant heterotroph in the oceans

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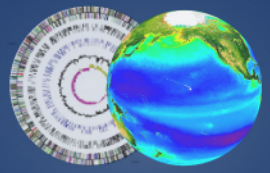

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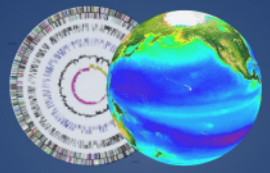
- Molecular microbial ecology/ribosomal RNA approach
- Characterization of rRNA genes from seawater, ca. 1990-2000
- Counting native SAR11 cells in seawater via FISH
- Ecotypes and the coarse structure of the SAR11 SSU rRNA tree
- Cultivation of SAR11 strains
- Comparative genomics of SAR11 isolates

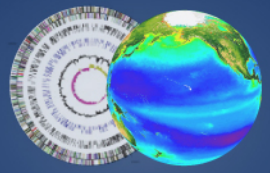


Molecular microbial ecology: the ribosomal RNA approach

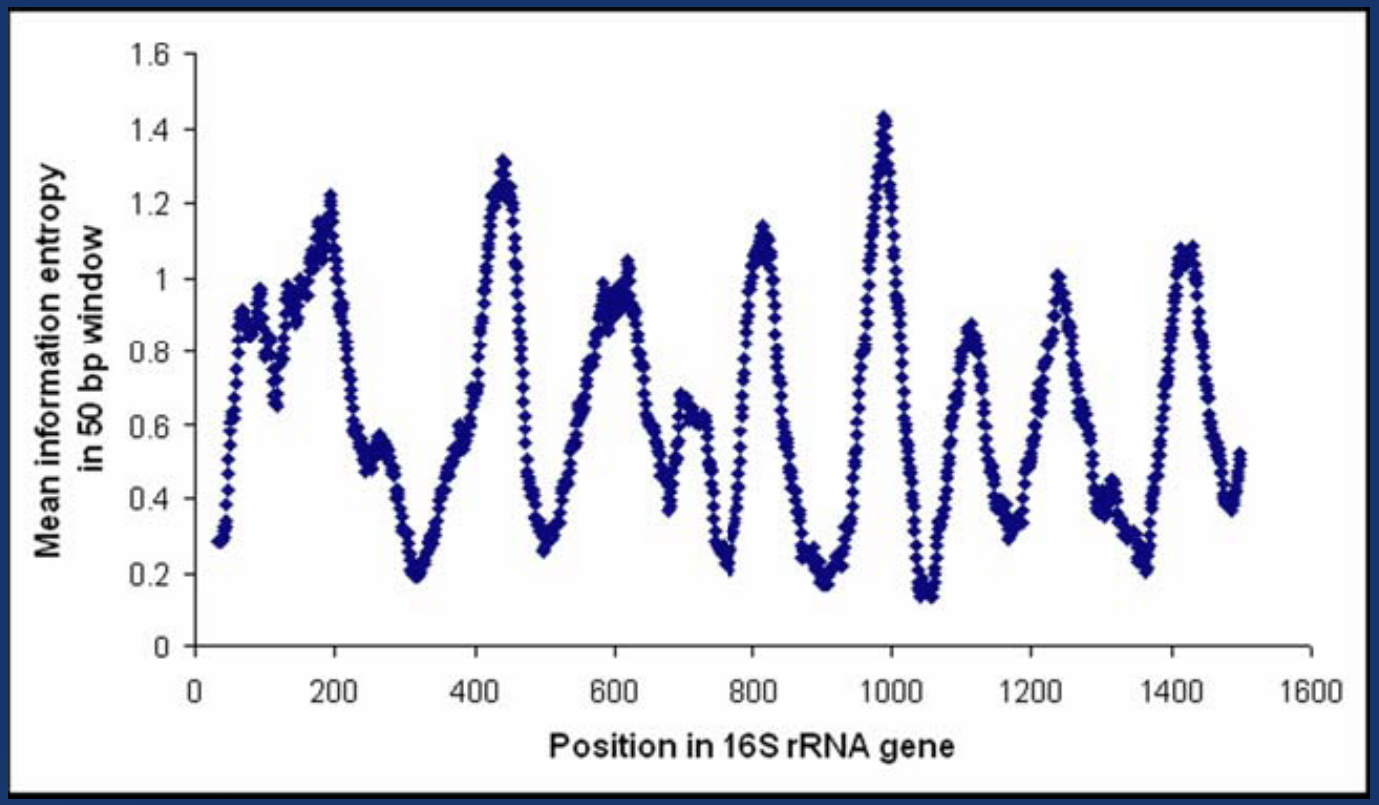
- Laboratory of Norm Pace (Pace et al. 1986) (Norm Pace, Dave Stahl, Gary Olsen, Steve Giovannoni, Ed DeLong, Tom Schmidt, David Lane, and others I am probably forgetting)
- Retrieve and sequence ribosomal RNAs from the natural environment to determine phylogenetic diversity of microorganisms present
- Started with 5S, quickly led to the small subunit (SSU or 16S) rRNA gene
- 16S rRNA gene sequences serve as phylogenetic markers for comparing microorganisms (cultured isolates and environmental gene clones)
- Note: SSU rRNA gene = 16S rRNA gene = 16S rDNA = SSU rDNA

Small subunit (SSU) rRNA gene

- Widely used phylogenetic marker for cultivated microorganisms
- Present in all organisms with a conserved function
  - 18S rRNA gene in eukaryotic organisms
  - 16S rRNA gene in prokaryotic organisms (*Bacteria* and *Archaea*)
- With very few exceptions, is not laterally transferred
- Encodes for the SSU rRNA molecule that is a functional component of the ribosomal complex
  - No codon degeneracy
  - Fairly large (ca. 1500 bp) mosaic of highly conserved and highly variable regions – broad range of utility in phylogenetic analyses
- Conserved regions are useful PCR priming sites
- Substitutions are considered to be neutral



Variability along the 16S rRNA gene



Andersson et a. 2008 PLoS ONE

Phylogenetic conservation superimposed onto the *E. coli* SSU rRNA secondary structure

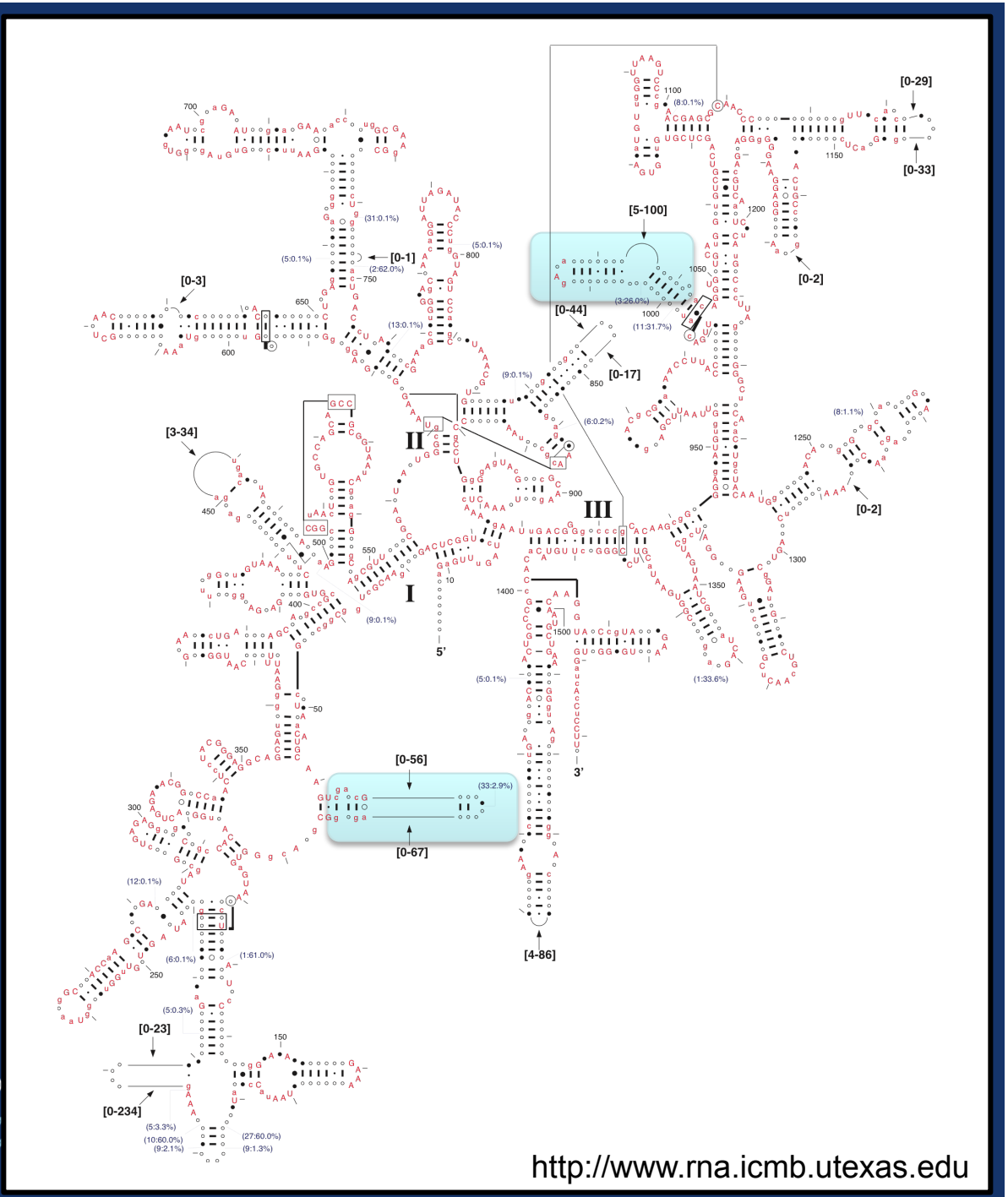
Number of sequences: 4214

ACGU 98+% conserved

acgu 90-98% conserved

• 80-90% conserved

o <80% conserved



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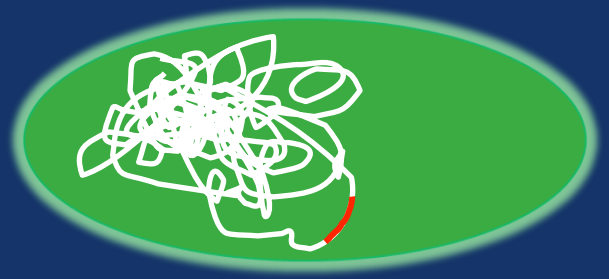
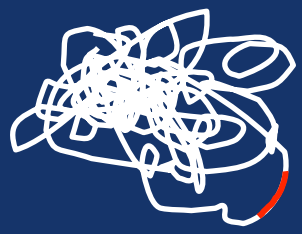
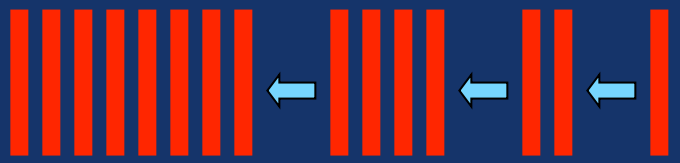
Identification of an isolated bacterial strain via 16S rRNA gene sequencing



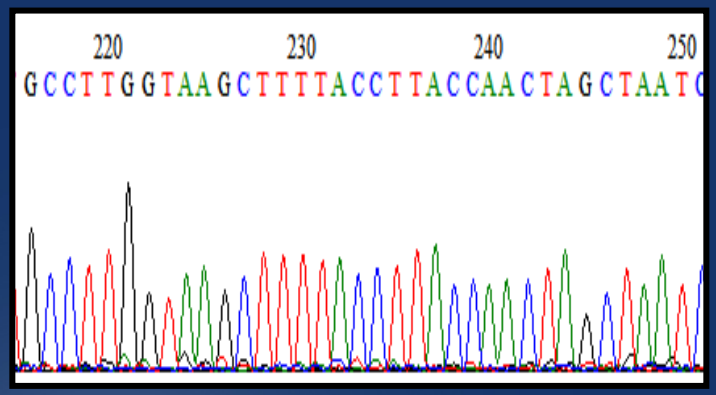
Extract genomic DNA

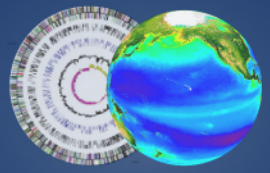


PCR amplify
16S rRNA gene



Sequence
PCR amplicon





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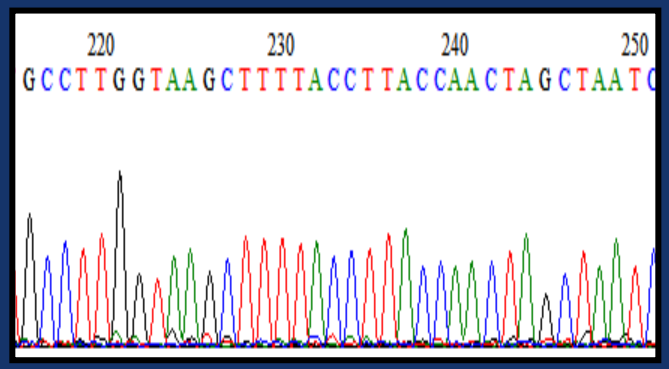
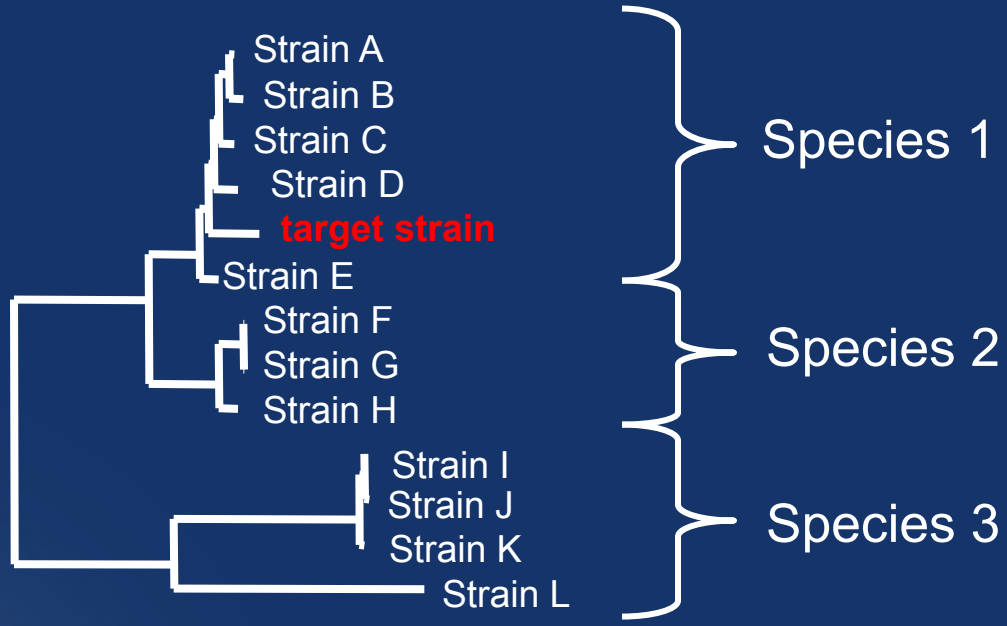
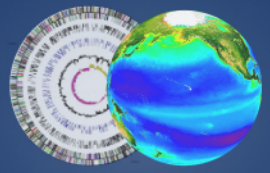


Align with database

Strain A	AGCGCTTTGATGTTC
Strain B	AGCGCTTTGATGTTC
Strain C	AGCGCTTTGATGTTC
Target	AGAGCTCTGATGCTC
Strain D	AGAGCTCTGATGCTC

Calculate evol. distances and build tree





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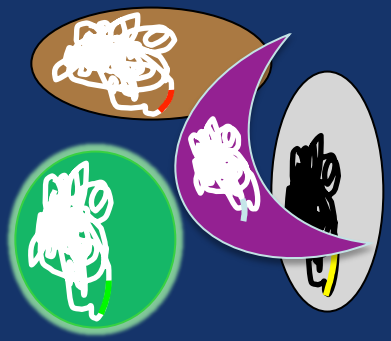
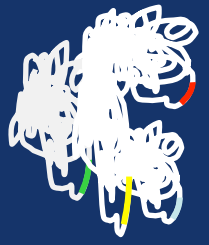
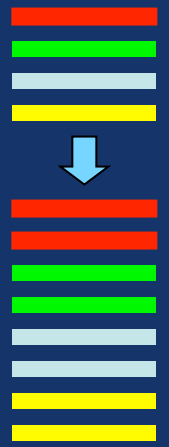
What if you had a mixed microbial community?



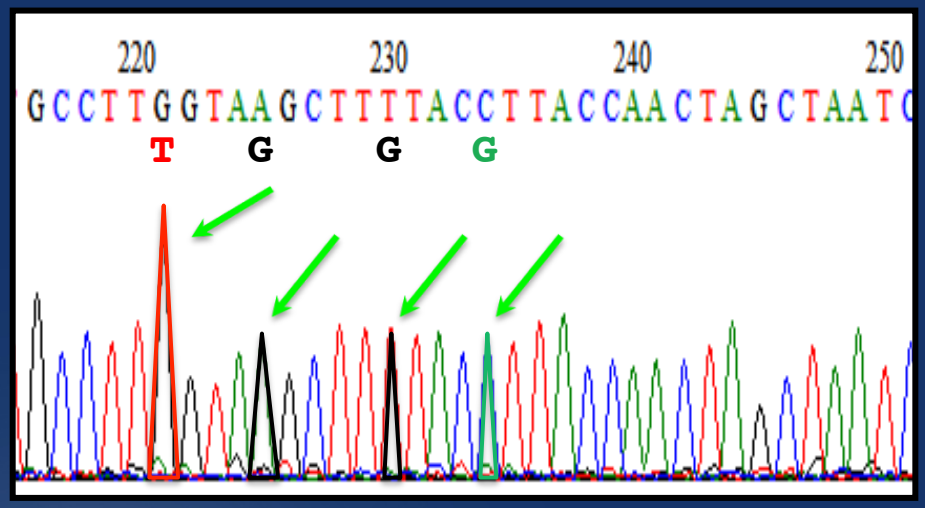
Extract genomic DNA

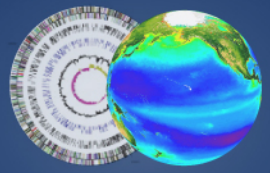


PCR amplify 16S rRNA gene



Sequence PCR amplicon





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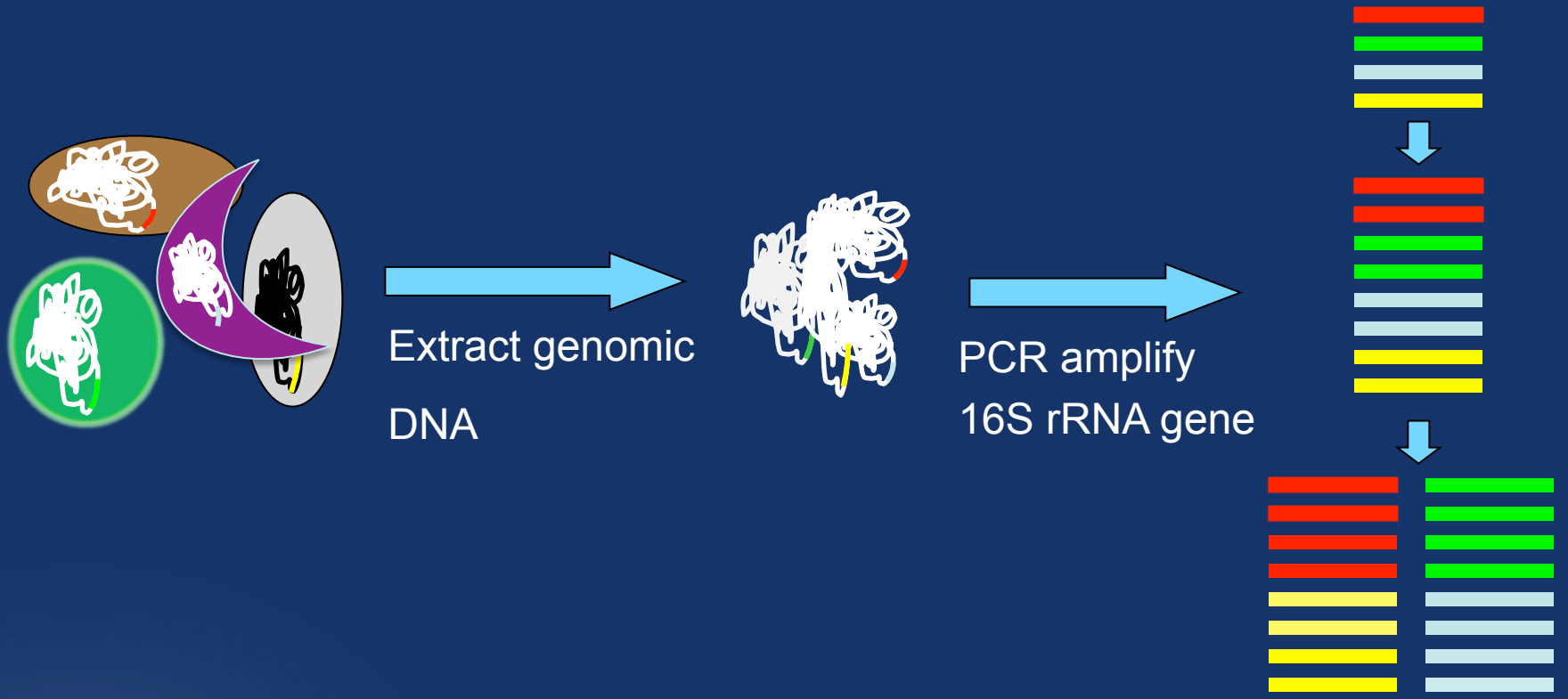
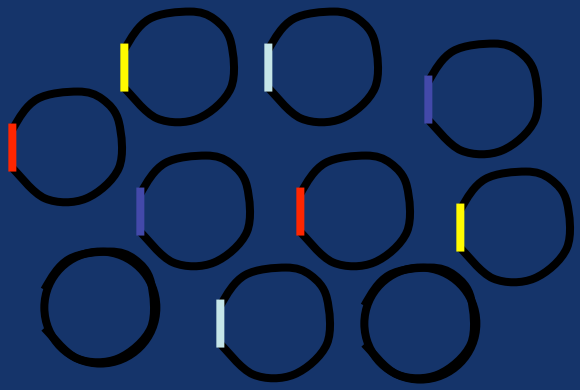
RNA gene cloning and sequencing from a mixture of microorganisms

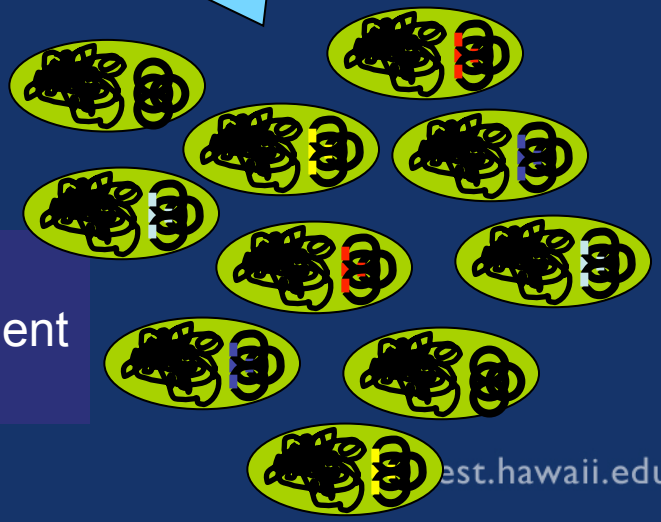


RNA gene cloning and sequencing from a mixture of microorganisms

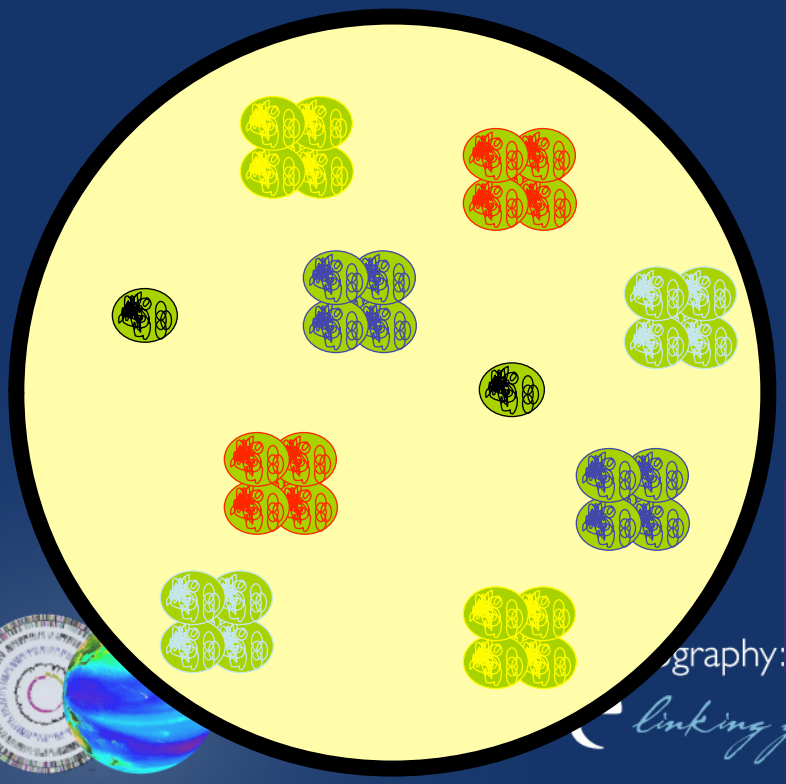
Ligate PCR product into plasmid vector



Transform E. coli cells with plasmid



Spread E. coli cells onto nutrient agar plate



graphy:

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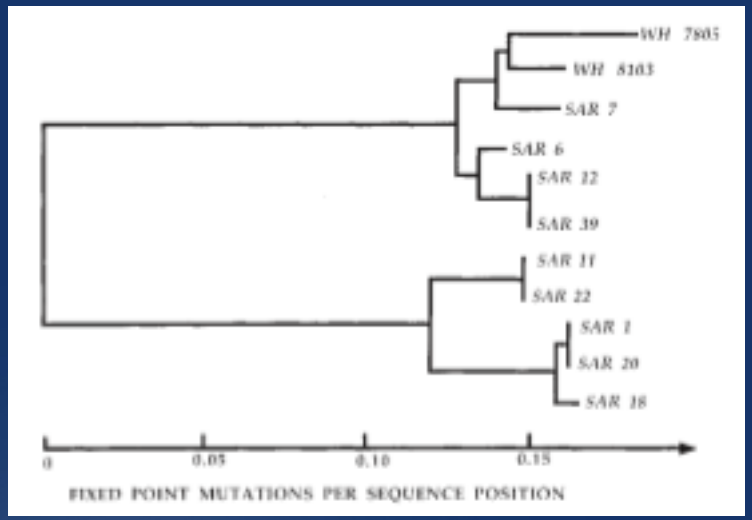
Genetic diversity in Sargasso Sea bacterioplankton

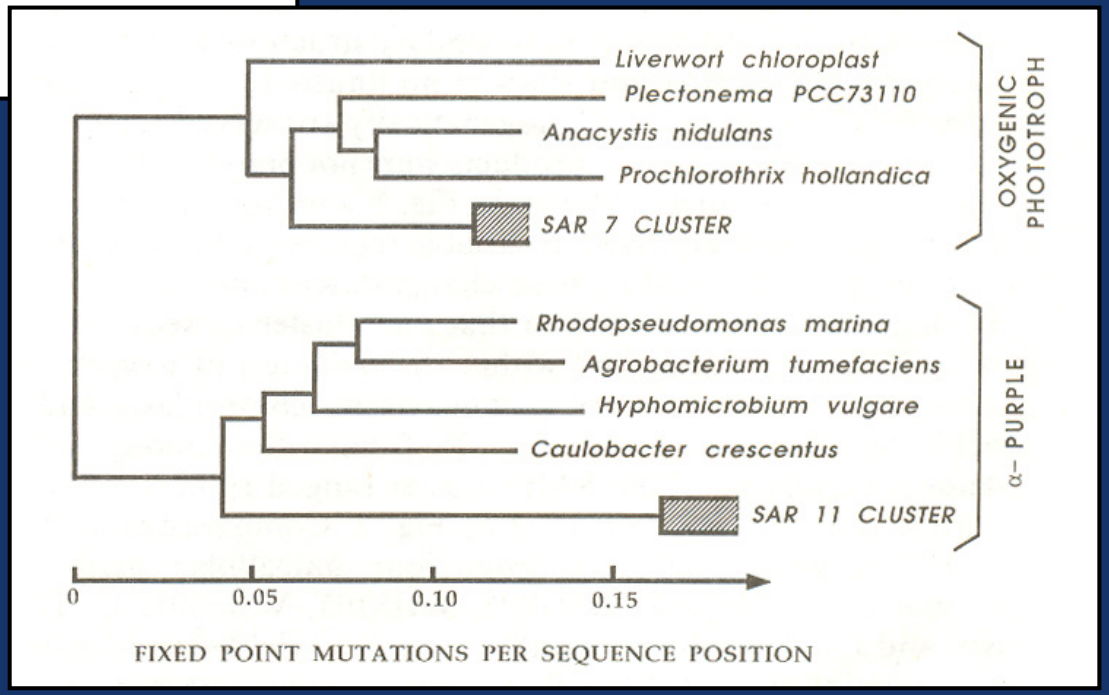
Stephen J. Giovannoni, Theresa B. Britschgi, Craig L. Moyer, & Katharine G. Field

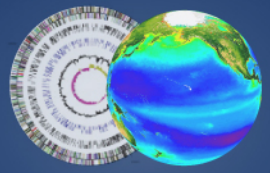
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331, USA

Nature **345**, 60 - 63 (03 May 1990)

First study to use the polymerase chain reaction (PCR) to amplify 16S rRNA genes from a natural environment (Sargasso Sea surface seawater)







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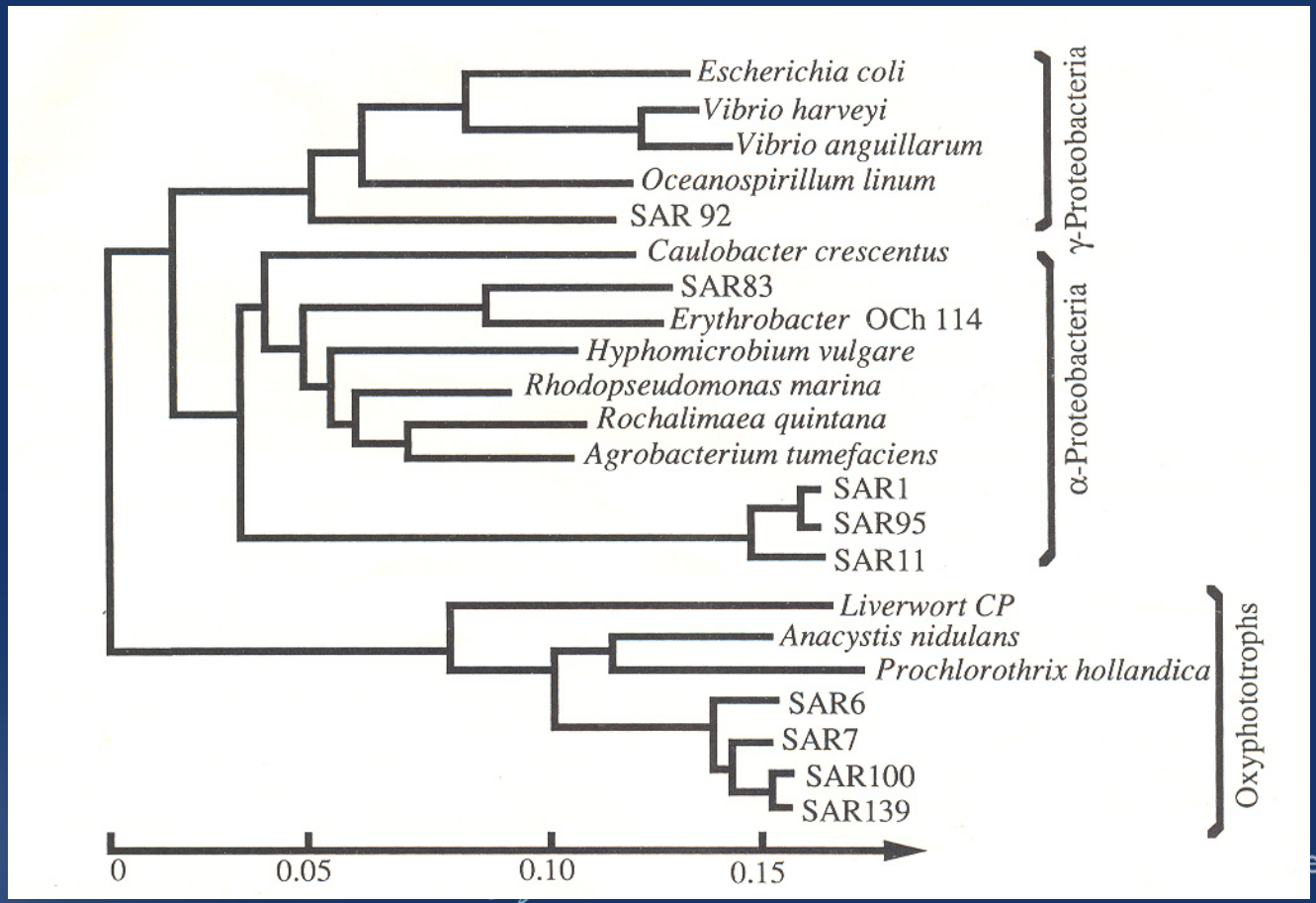
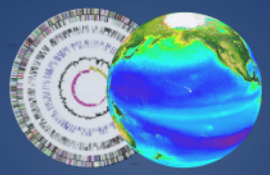
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1991, p. 1707-1713 Vol. 57, No. 6

Phylogenetic Analysis of a Natural Marine Bacterioplankton Population by rRNA Gene Cloning and Sequencing

THERESA B. BRITSCHGI AND STEPHEN J. GIOVANNONI*

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331



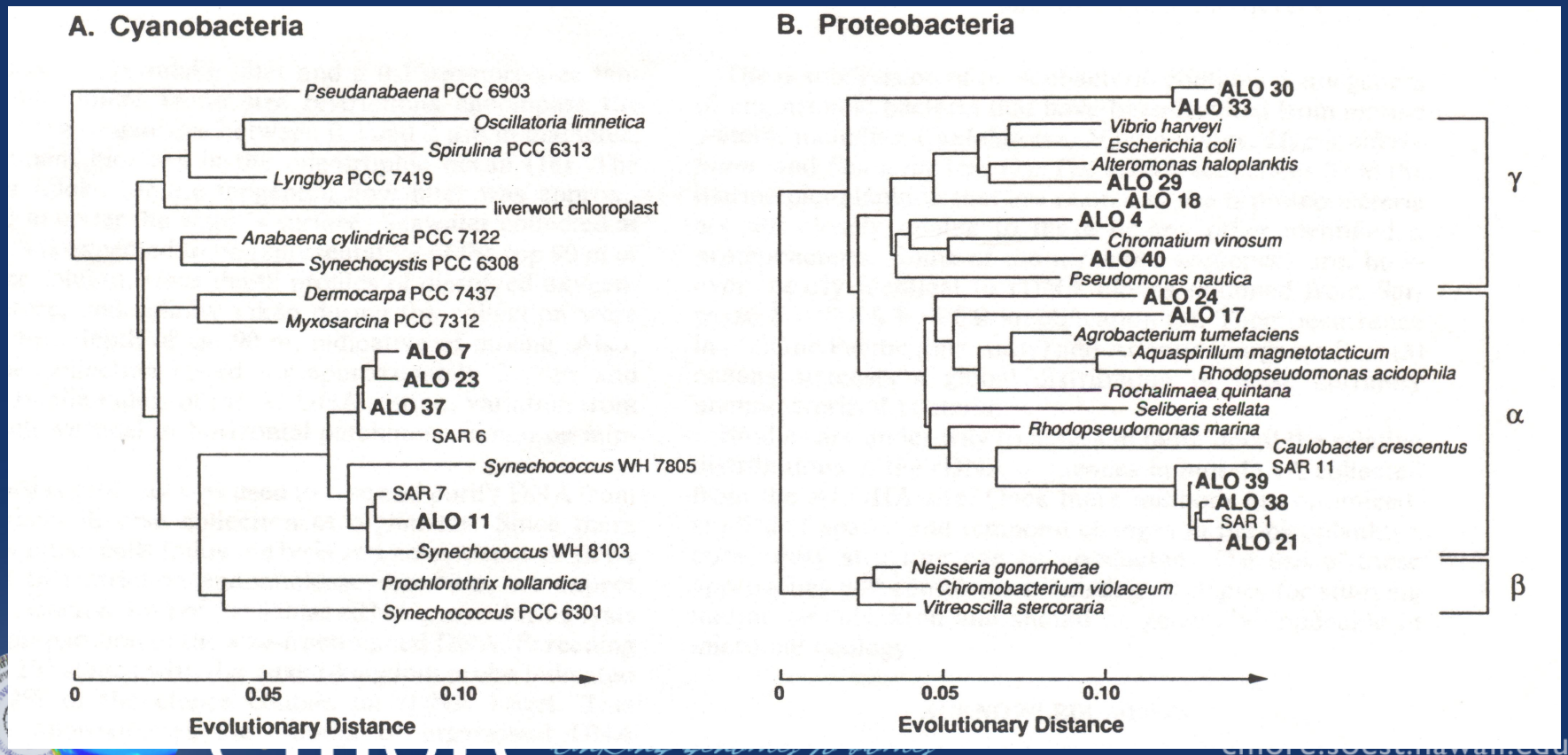


JOURNAL OF BACTERIOLOGY, July 1991, p. 4371-4378 Vol. 173, No. 14

Analysis of a Marine Picoplankton Community by 16S rRNA Gene Cloning and Sequencing

THOMAS M. SCHMIDT, EDWARD F. DELONG, AND NORMAN R. PACE*

Department of Biology and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, Indiana 47405



Summary of 16S rRNA gene clone libraries from marine prokaryotic plankton, ca. 1999

Clone library prefix	Seawater source (depth, location)	Method of construction	Clones analyzed[1]	Refs.
SAR1-199	surface water, Sargasso Sea, Atlantic Ocean	PCR-bacterial[2]	44	Giovannoni et al. 1990, Britschgi & Giovannoni 1991, Mullins et al. 1995
ALO	surface water, north central Pacific Ocean	Shotgun clone library	15	Schmidt et al. 1991
SBAR	surface water, Santa Barbara Channel, Pacific Ocean	PCR-archaeal	20	DeLong 1992
WHAR	surface water, Woods Hole, east coast of U.S., Atlantic Ocean	PCR-archaeal	20	DeLong 1992
NH25	100 m, northeastern Pacific Ocean	PCR-universal	13	Fuhrman et al. 1992, Fuhrman et al. 1993
NH49	500 m, northeastern Pacific Ocean	PCR-universal	10	Fuhrman et al. 1992, Fuhrman et al. 1993
NH16	100 m, northeastern Pacific Ocean	PCR-universal	15	Fuhrman et al. 1993
NH29	100 m, northeastern Pacific Ocean	PCR-universal	9	Fuhrman et al. 1993
BDA1	10 m, Sargasso Sea, Atlantic Ocean	PCR-universal	14	Fuhrman et al. 1993
FL	surface water, Santa Barbara Channel, Pacific Ocean	PCR-bacterial	20	DeLong et al. 1993
ANTARCTIC	surface water, Arthur Harbor, Antarctica	PCR-archaeal	14	DeLong et al. 1994
OAR	surface water, Oregon coast, Pacific Ocean	PCR-archaeal	--[3]	DeLong et al. 1994
SAR400-599	80 m, Sargasso Sea, Atlantic Ocean	PCR-bacterial	92	Gordon & Giovannoni 1996, Field et al. 1997
SAR200-399	250 m, Sargasso Sea, Atlantic Ocean	PCR-bacterial	113	Giovannoni et al. 1996, Wright et al. 1997, Field et al. 1997
OCS	10 m, Oregon coast, Pacific Ocean	PCR-bacterial	114	Suzuki et al. 1997, Rappé et al. 2000
SB95	surface and 200 m, Santa Barbara Channel, Pacific Ocean	PCR-archaeal	575	Massana et al. 1997
OM	10 m, eastern continental shelf of U.S., Atlantic Ocean	PCR-bacterial	169	Rappé et al. 1997
p712	500 m, Pacific Ocean basin	PCR-universal	18	Fuhrman & Davis 1997
pN1	3000 m, Pacific Ocean basin	PCR-universal	23	Fuhrman & Davis 1997
pB1	1000 m, Atlantic Ocean basin	PCR-universal	21	Fuhrman & Davis 1997
C	10 m, west Irish coast, Atlantic Ocean	PCR-archaeal	7	McInerney et al. 1997
PM	0, 100, and 500 m, northeast Altantic Ocean	PCR-archaeal	3	McInerney et al. 1997
pC2	surface water, Long Island Sound, New York, Atlantic Ocean	PCR-universal	17	Fuhrman & Ouverney 1998
pC8	surface water, Long Island Sound, New York, Atlantic Ocean	PCR-universal	7	Fuhrman & Ouverney 1998
pM	surface water, Malibu Pier, California, Pacific Ocean	PCR-universal	16	Fuhrman & Ouverney 1998
pM9	surface water, Monterey Bay, California, Pacific Ocean	PCR-universal	5	Fuhrman & Ouverney 1998

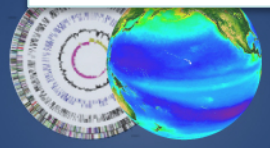
n = 6 bacterial (552), 7 archaeal (639), 13 universal (183, mostly bacteria)

Giovannoni & Rappé 2000

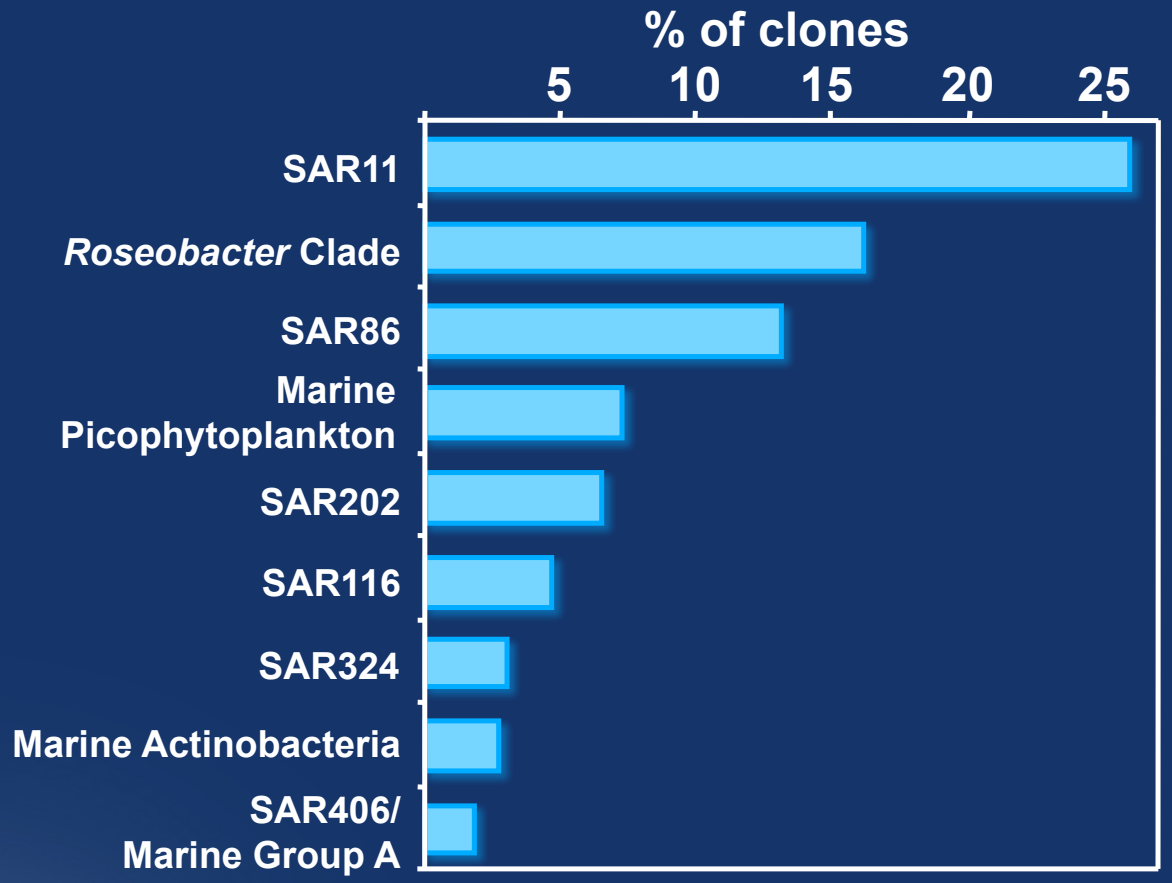
Distribution of major bacterial SSU rRNA gene clone clusters in libraries from marine prokaryotic plankton, ca. 1999

Clone library prefix	SAR11	SAR83	SAR86	SAR116	marine Pico.	marine Actino.	SAR202	SAR324	SAR406
SAR (0m)	X	X	X	X	X	X			
ALO	X	X	X	X	X				
NH25	X				X	X			
NH49	X		X						X
NH16	X	X	X		X	X			X
NH29	X		X		X	X			
BDA1	X		X		X	X			
FL	X								
SAR (80m)	X	X	X	X	X	X			X
SAR (250m)	X	X					X	X	
OCS	X	X	X	X		X			X
OM	X	X	X	X		X			
p712	X							X	X
pN1	X		X				X	X	X
pB1	X						X	X	X
pC2	X	X							
pC8	X	X		X					
pM	X	X	X						
pM9	X				X				

Giovannoni & Rappé 2000



Relative abundance of the major bacterial rRNA gene clone groups in seawater



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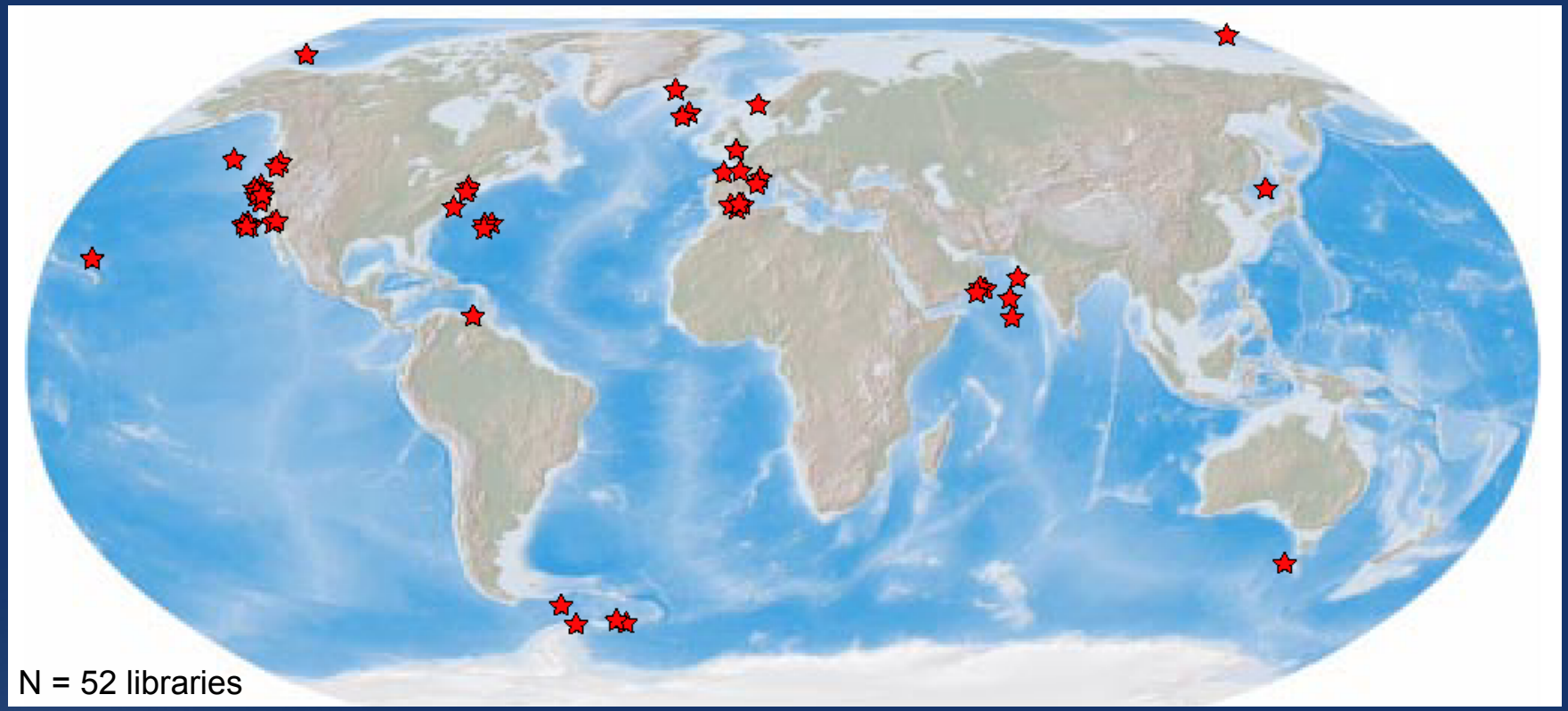
Giovannoni & Rappé 2000

Distribution of SAR11 SSU rRNA gene clones in seawater of the global ocean, ca. 2002



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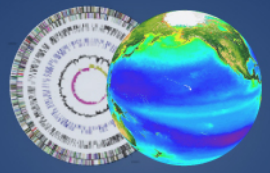
Morris et al. 2002

Fluorescence *in situ* hybridization (FISH)

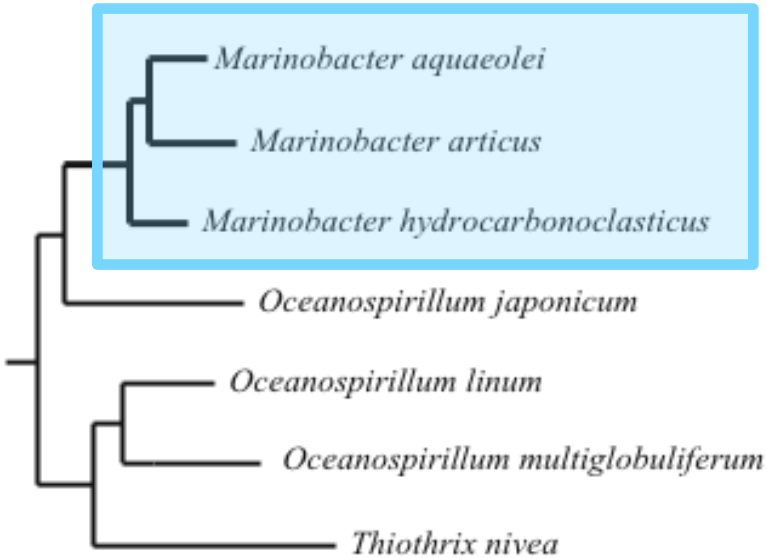
- The use of fluorescently labeled oligonucleotide probes to detect specific SSU rRNA molecules inside intact microbial cells
- Idea is that the cells that harbor specific SSU rRNA gene phylotypes (e.g. SAR11, marine Archaea) can be quantified in seawater samples to yield absolute abundance values

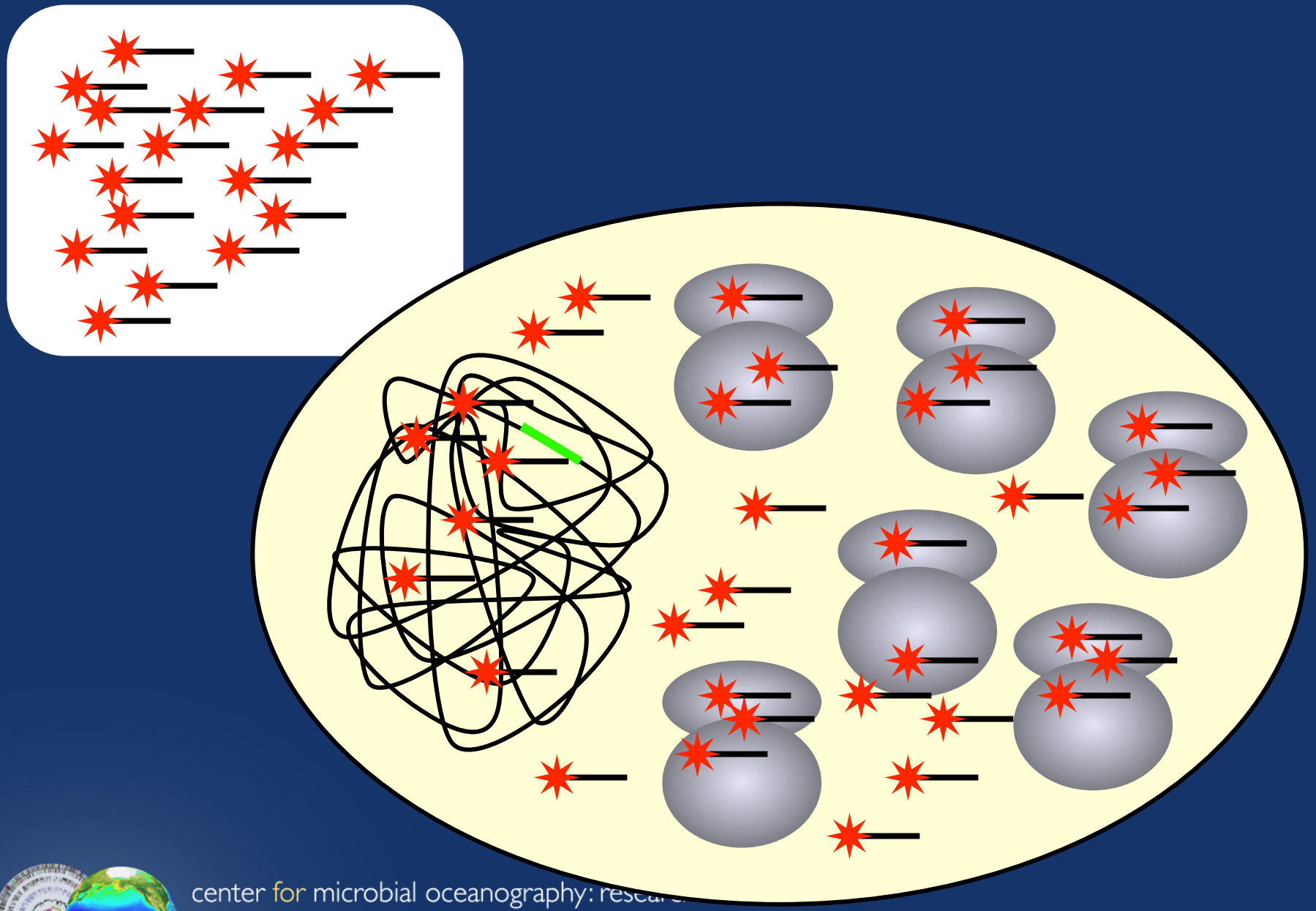
Giovannoni, S. J., E. F. DeLong, G. J. Olsen and N. R. Pace. 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. J. Bacteriol. 170: 720-726

DeLong E.F., G. Wickham, and N. R. Pace. 1989. Phylogenetic stains: Ribosomal RNA-based probes for identification of single microbial cells. Science 243:1360-1363.



Probe design: an example





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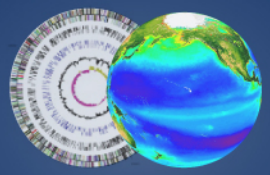
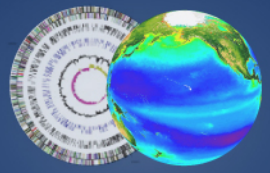
How do you increase fluorescence signal per cell?

- Increase probe accessibility to its target: e.g. use “helper” oligonucleotides or optimize position along the 16S rRNA
- Amplify signal strength from a single oligonucleotide probe (attach multiple fluorescent moieties or amplify via enzymatic reaction such as TSA)
- Use multiple probes that target the same group, but at different positions around the 16S rRNA
- Polynucleotide probes

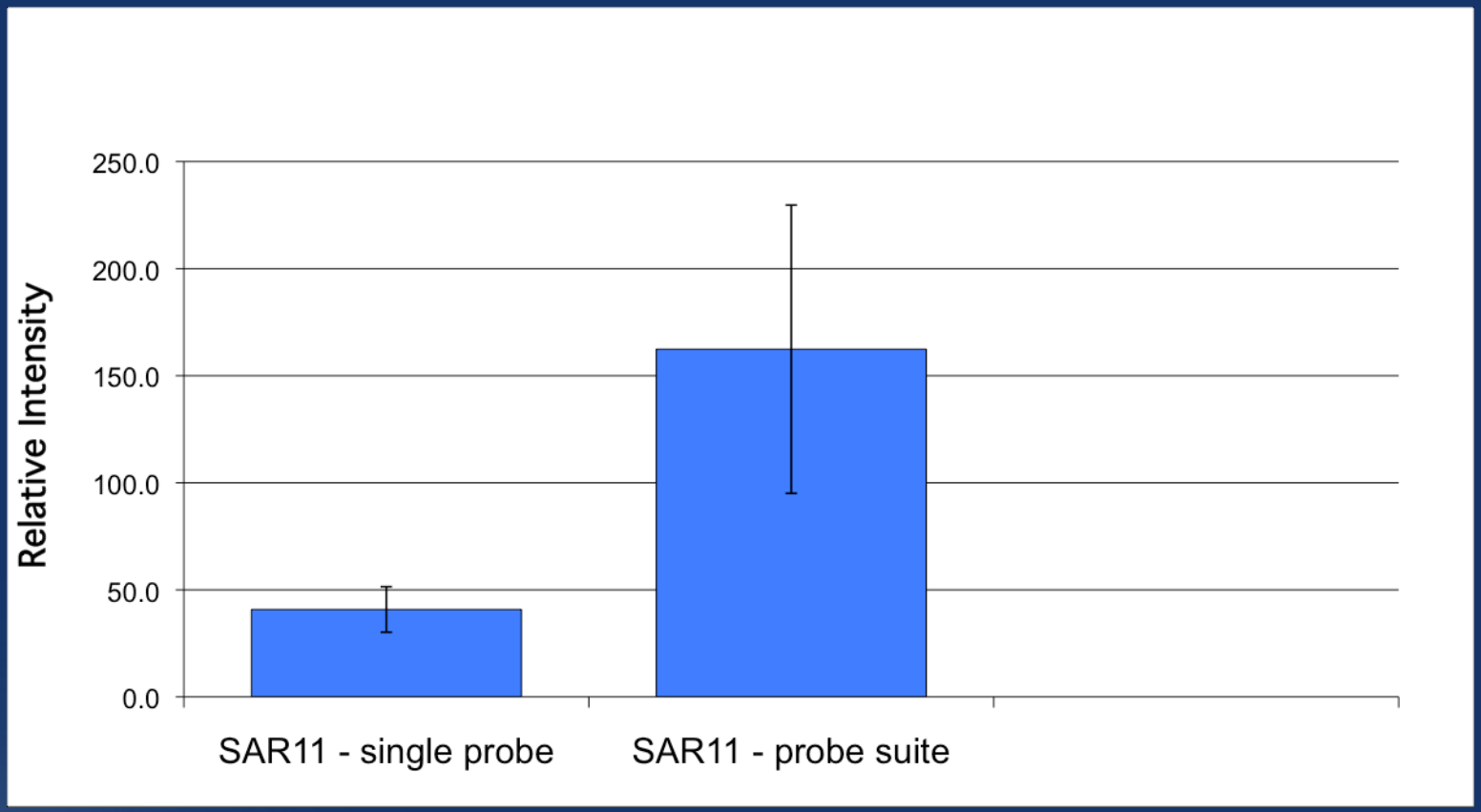


SAR11 probe suite

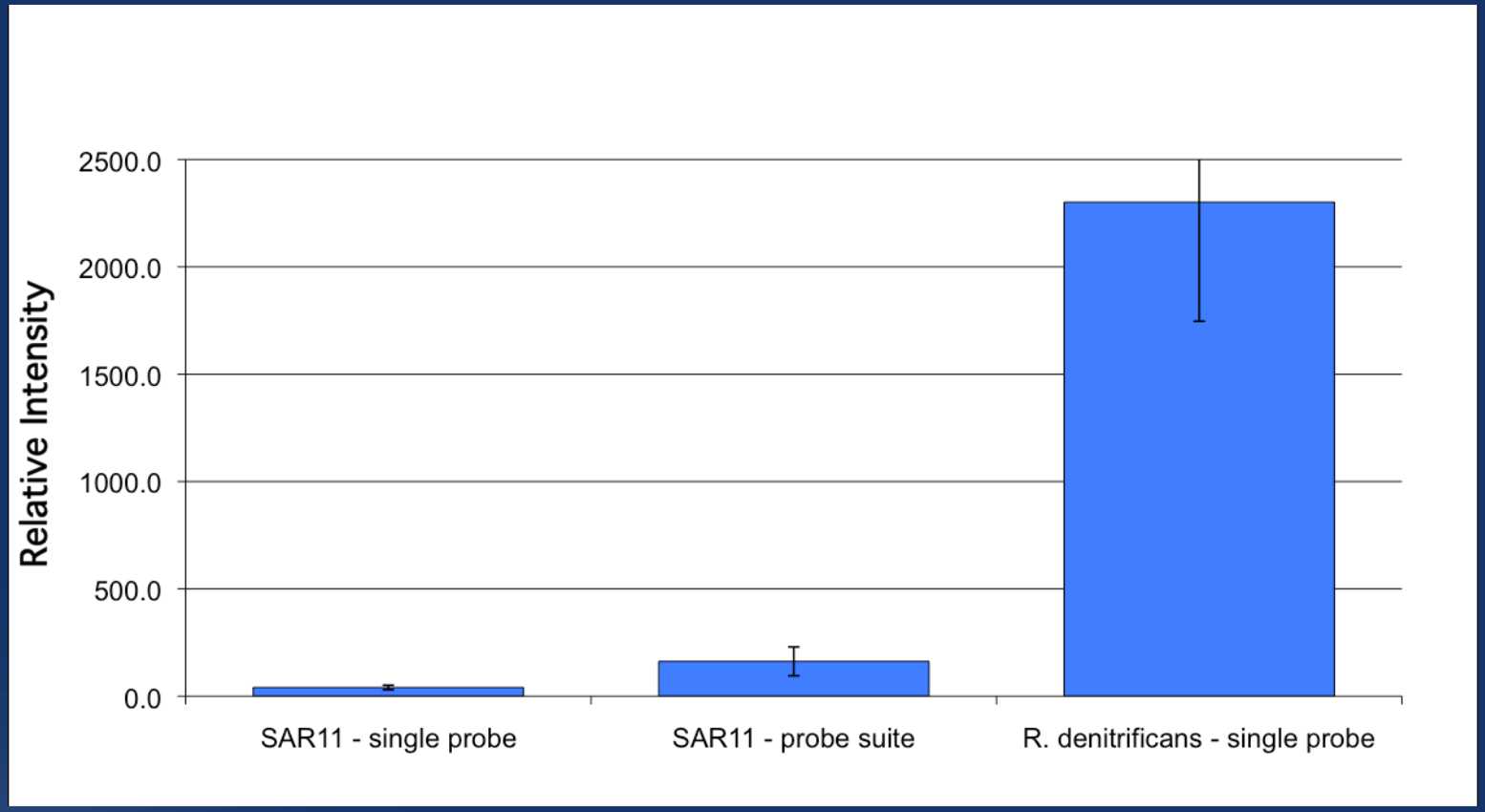
Probe ID	Probe Sequence (5'-3')	Location (*E. coli* numbering)
SAR11-152R	ATTAGCACAAGTTTCCYCGTGT	152-173
SAR11-441R	TACAGTCATTTTCTTCCCCGAC	441-487
SAR11-542R	TCCGAACTACGCTAGGTC	542-559
SAR11-732R	GTCAGTAATGATCCAGAAAGYTG	732-754



Fluorescence per native SAR11 cell - single oligonucleotide vs. probe suite

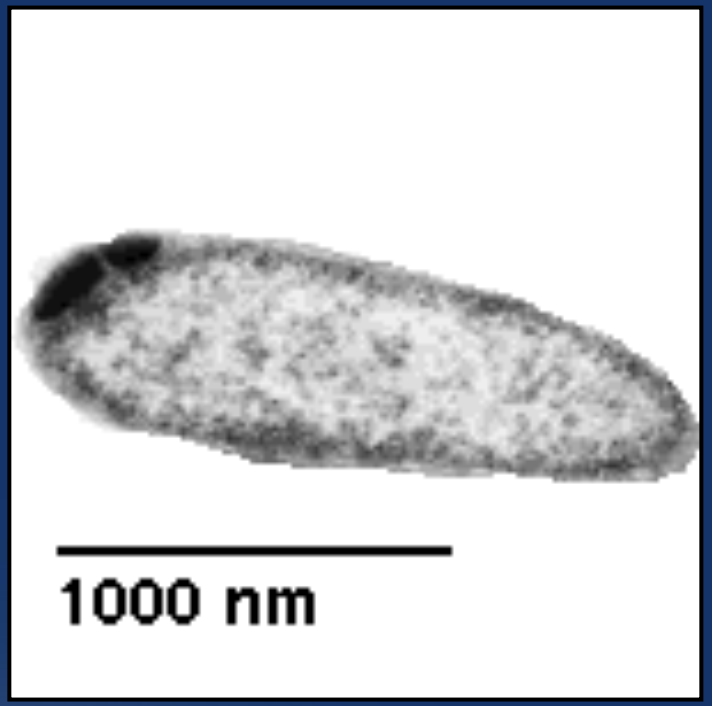


Comparison of fluorescent signals between native SAR11 cells and a culture of *Roseobacter denitrificans*



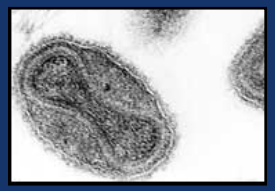
SAR11 cell size

E. coli
500 x 1500 nm



SAR11
200 x 400 nm

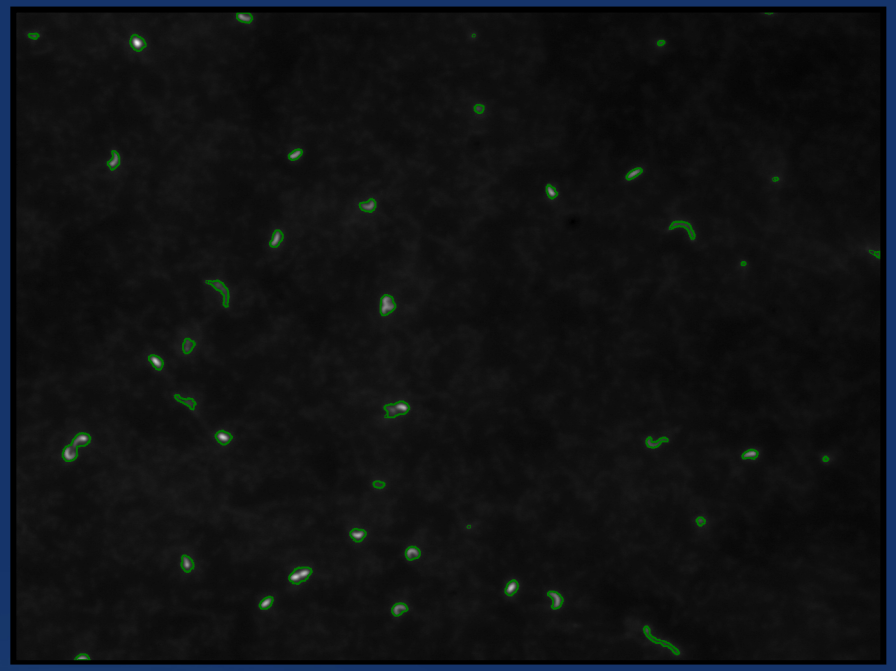
Vaccinia virus
200 x 300 nm



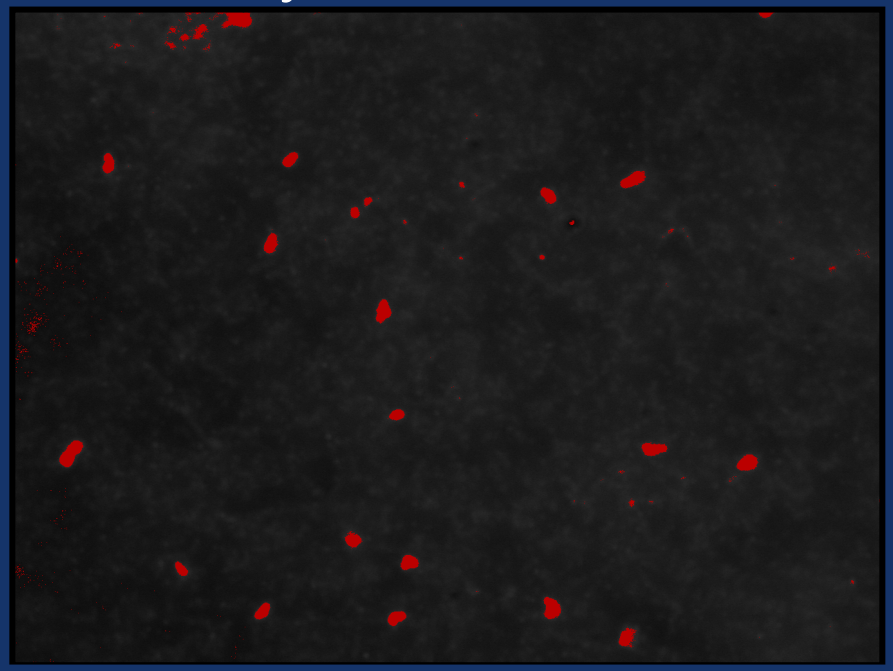
Minimum size estimate for the smallest free living cell: 200 to 300 nm in diameter

Counting SAR11 cells via FISH

DAPI-stained cells

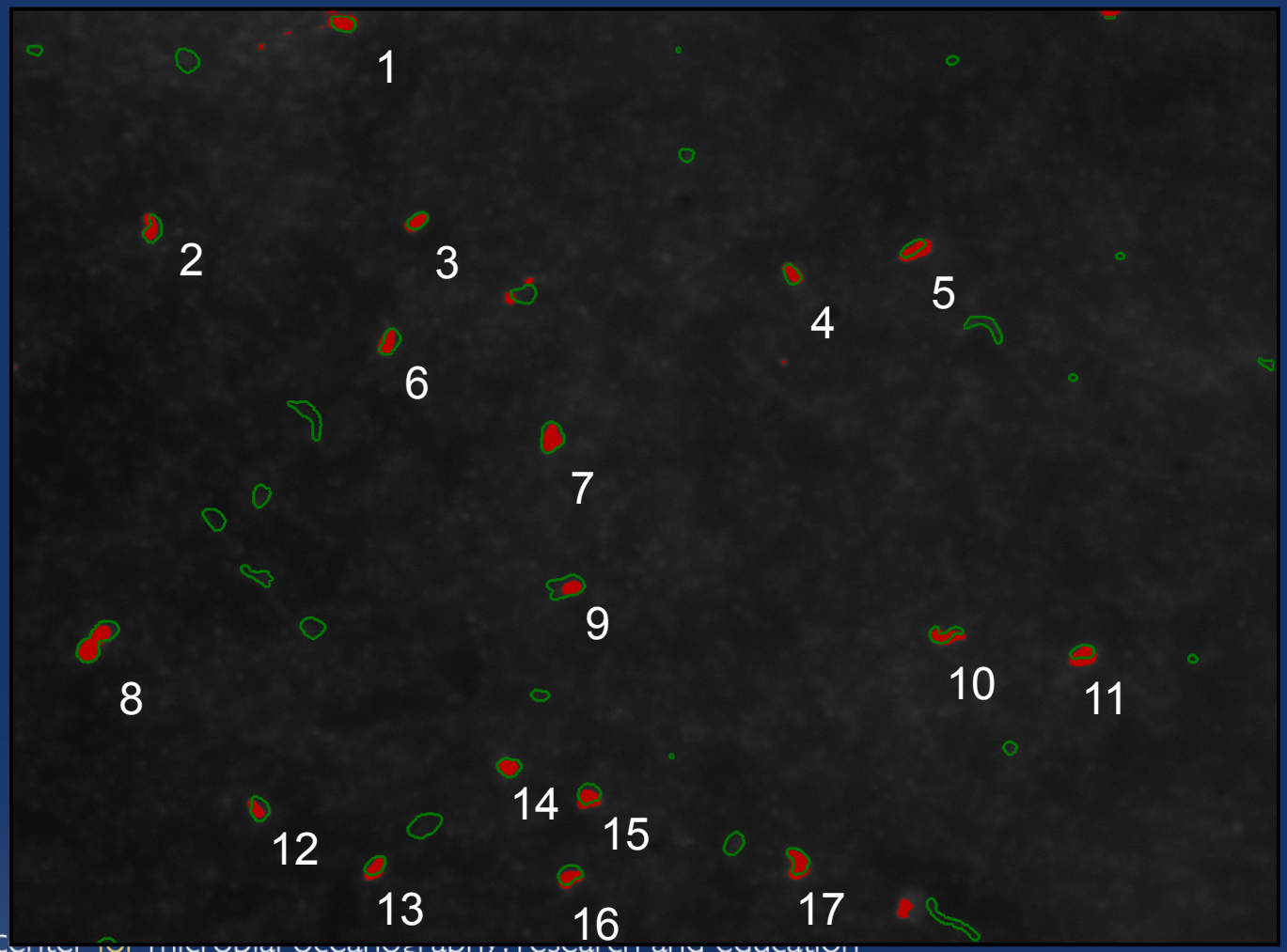


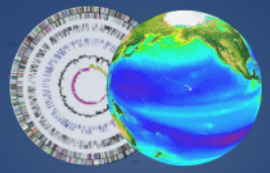
Hybridized cells



Segment corresponding DAPI and Cy3 images

Composite





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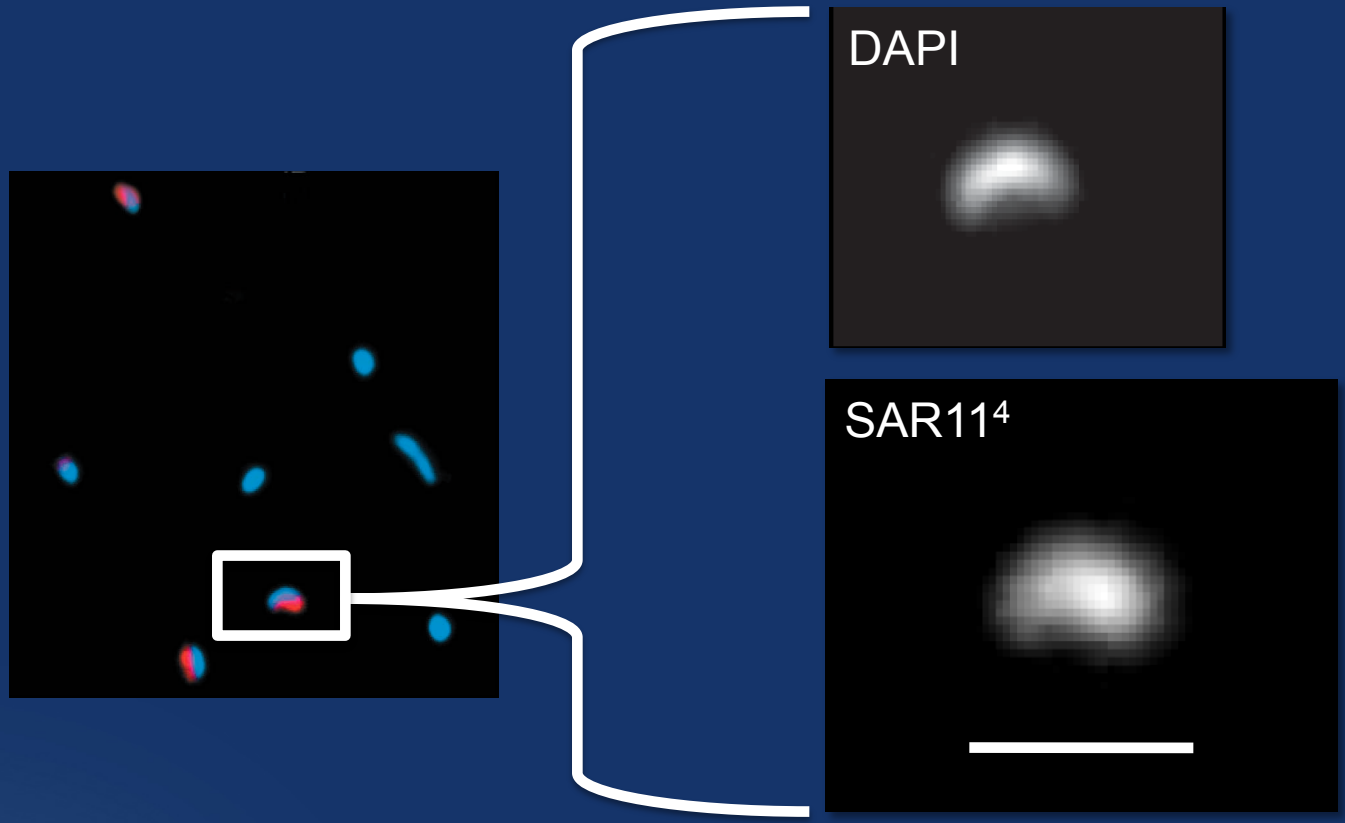
SAR11 FISH



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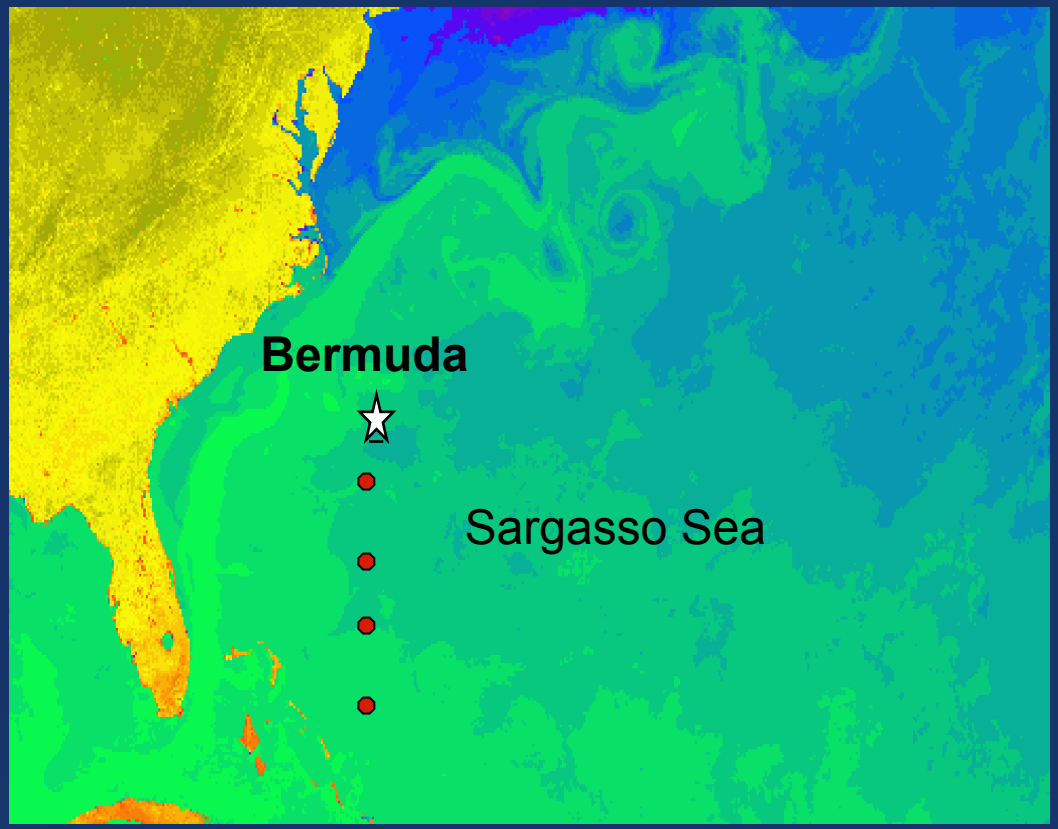
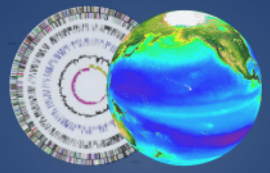
Morris et al. 2002

CDOM cruise, Atlantic Ocean, August 2001



Relative abundance of SAR11, CDOM cruise

Depth (m)	31 ºN	29 ºN	27 ºN	25 ºN	
1	35.1%	35.7%	36.0%	34.5%	
40	**51.2%**	**42.5%**	36.3%	33.4%	
60	34.1%	**40.6%**	**40.7%**	37.3%	
80	29.7%	31.9%	35.0%	32.6%	**35%**
100	34.3%	31.3%	33.7%	31.8%	
150	26.1%	36.1%	32.6%	31.3%	
250	25.1%	18.7%	10.7%	16.9%	
500				17.2%	**18%**
1000				19.0%	
3000				19.5%	



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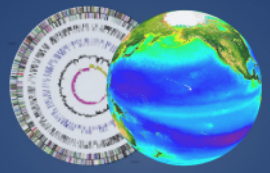
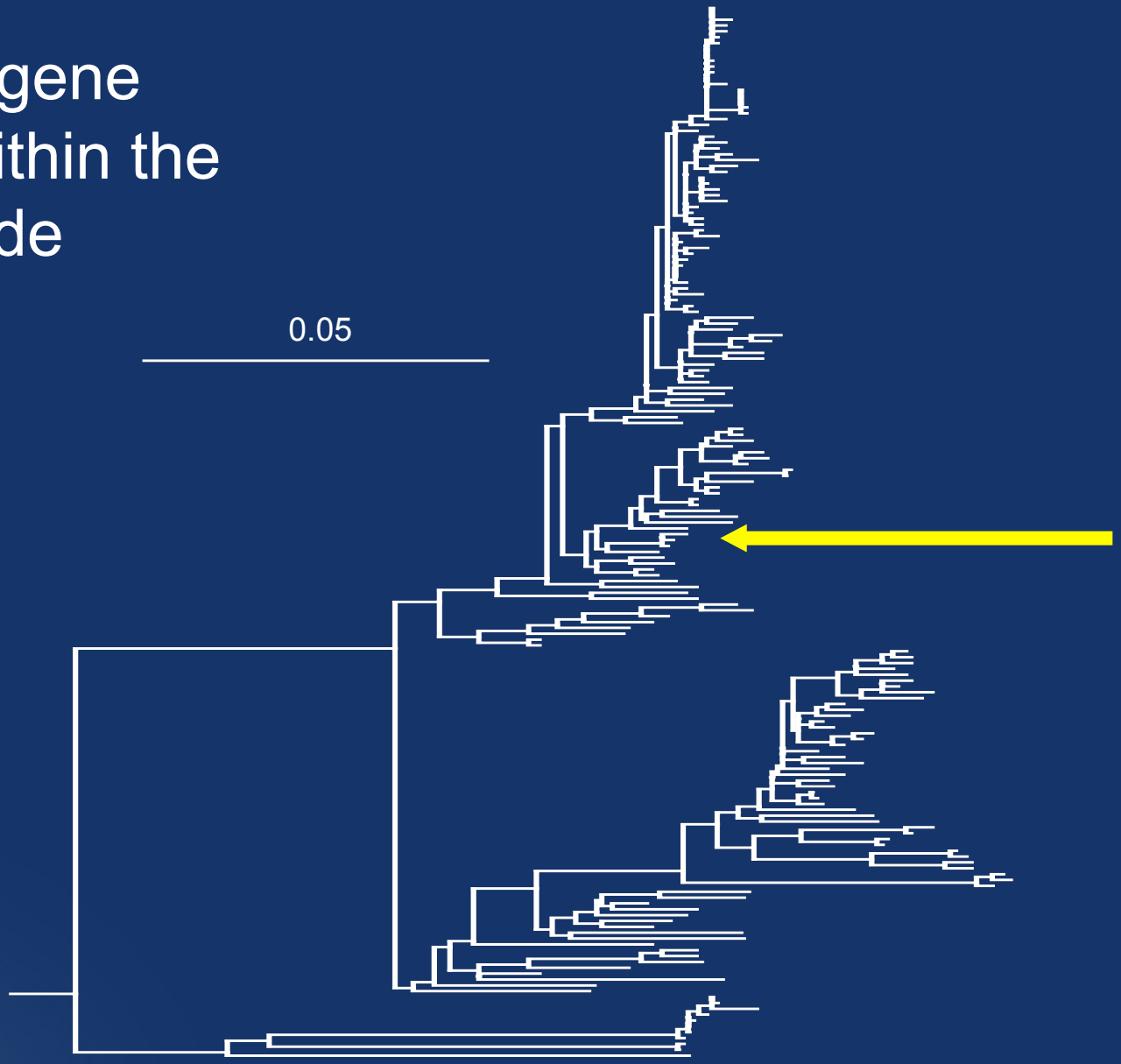
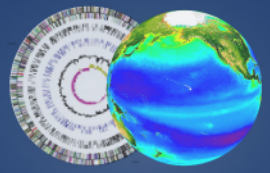
Sequence Diversity Within Major Marine Bacterioplankton 16S rRNA Gene Clusters

Group	Identity (all overlapping)[a]	Identity (conserved)[b]
SAR11	0.872	0.887
SAR86	0.935	0.945
SAR116	0.897	0.908
Roseobacter	0.884	0.904
SAR324	0.897	0.922
Marine Actinobacteria	0.940	0.966
Marine Picophytoplankton	0.962	0.969
SAR406/Group A	0.946	0.968



16S rRNA gene diversity within the SAR11 clade





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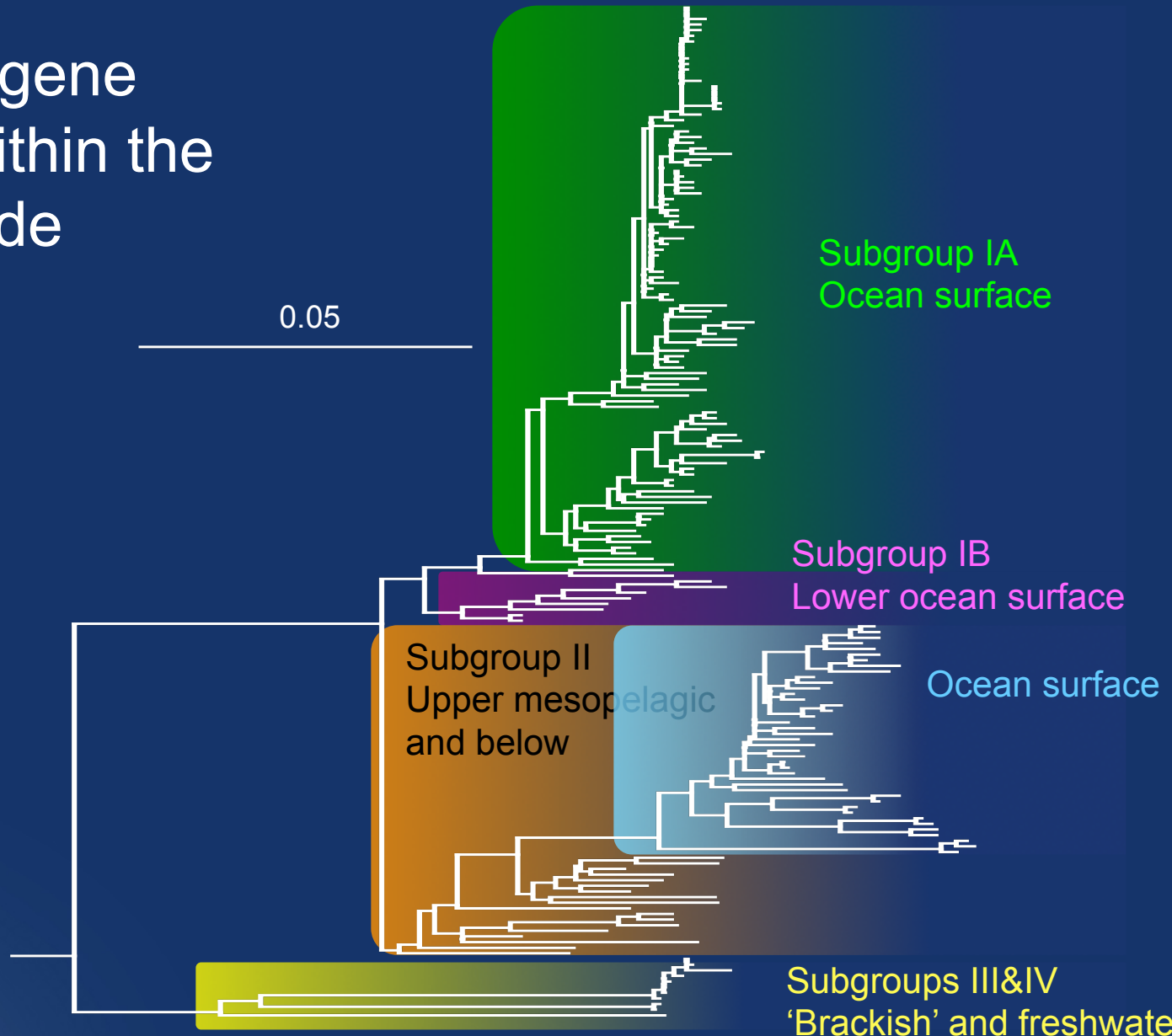
16S rRNA gene diversity within the SAR11 clade



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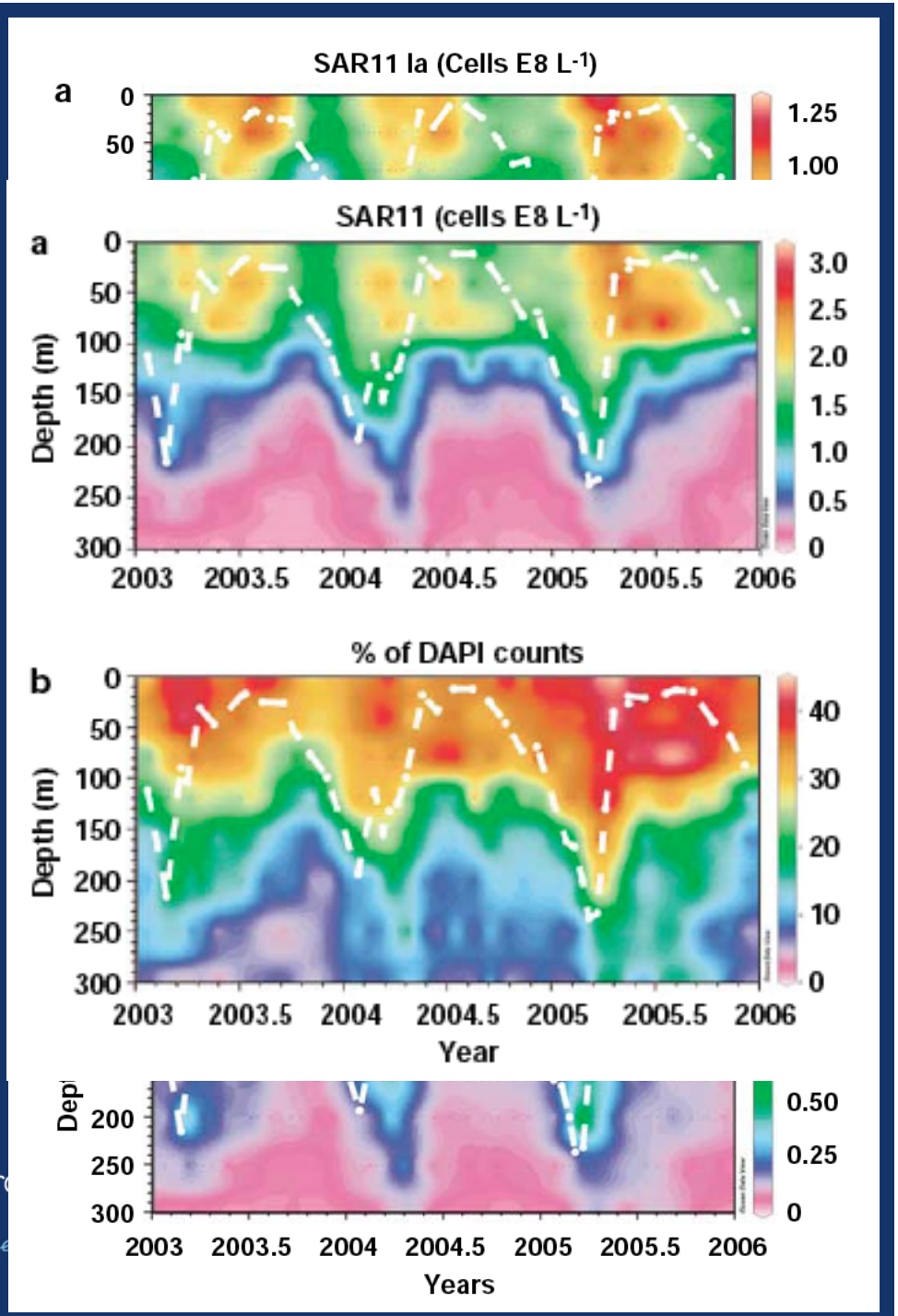
Seasonal dynamics of SAR11 populations in the euphotic and mesopelagic zones of the northwestern Sargasso Sea

Craig A Carlson, Robert Morris, Rachel Parsons, Alexander H Treusch, Stephen J Giovannoni and Kevin Vergin

The ISME Journal (2009) **3,** 283–295



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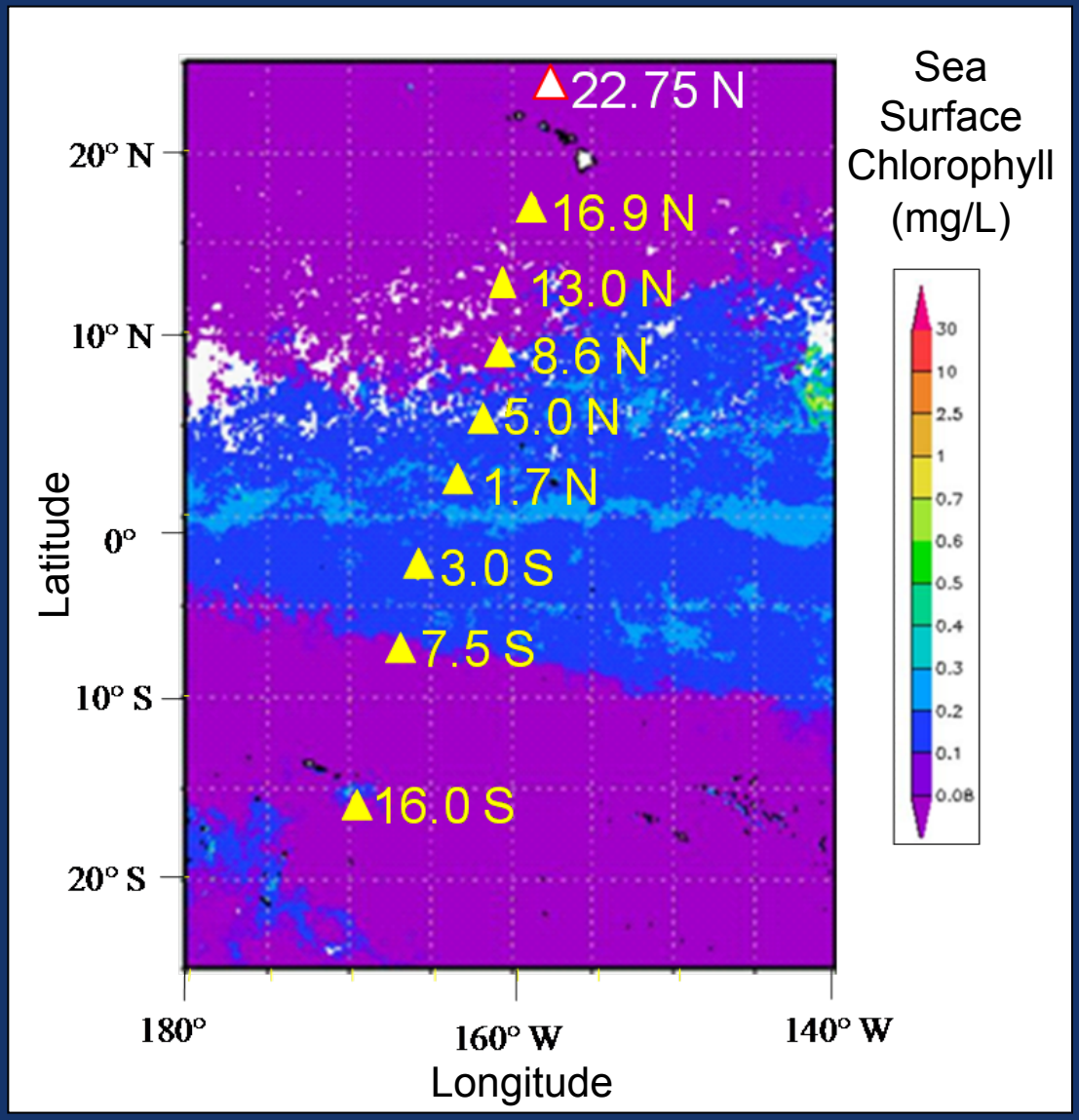
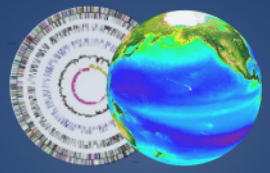
Biogeochemistry of the Upper Ocean: Latitudinal Assessment (BULA cruise)





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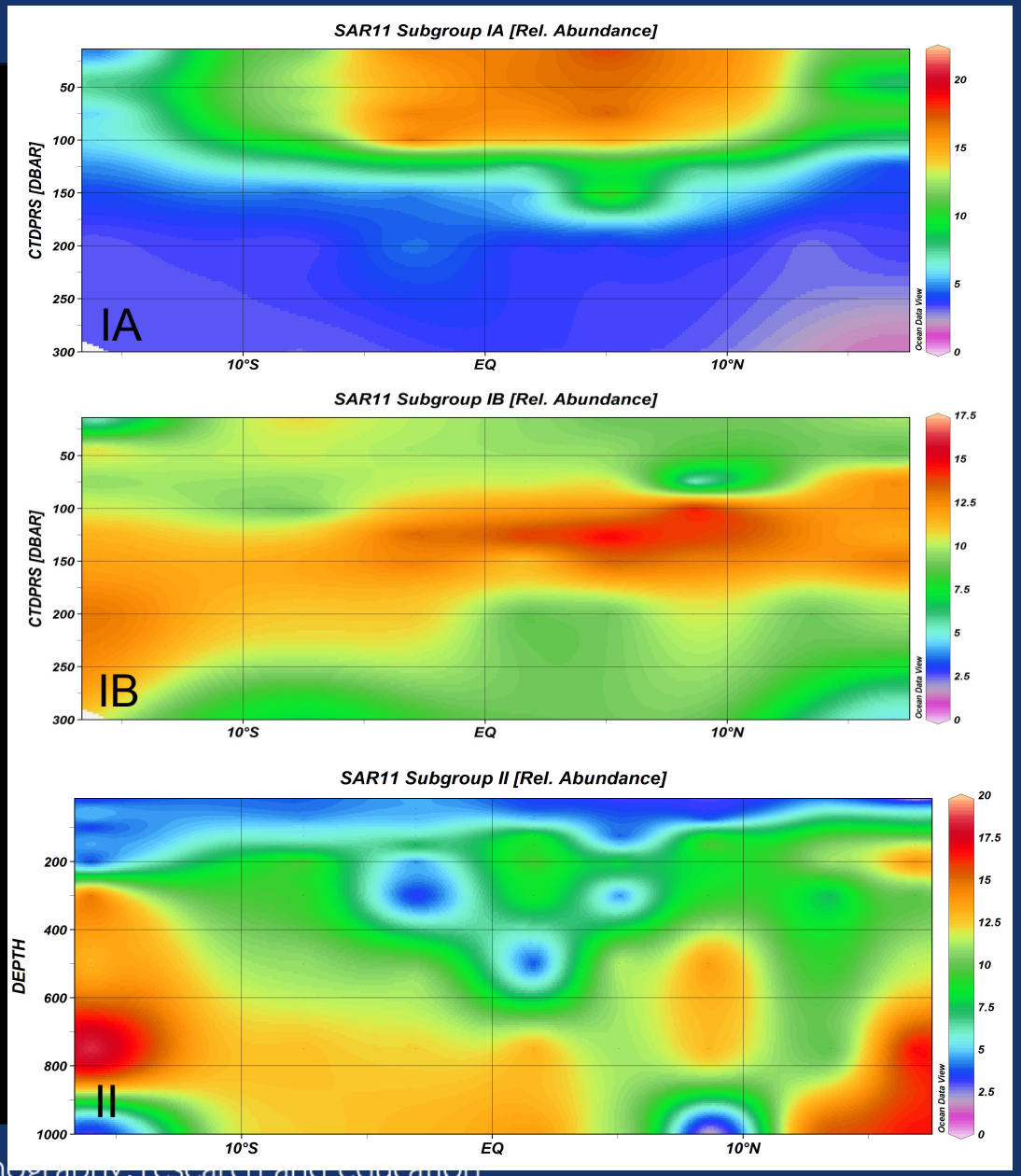

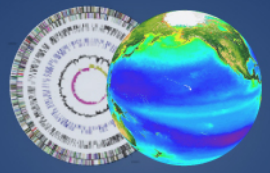

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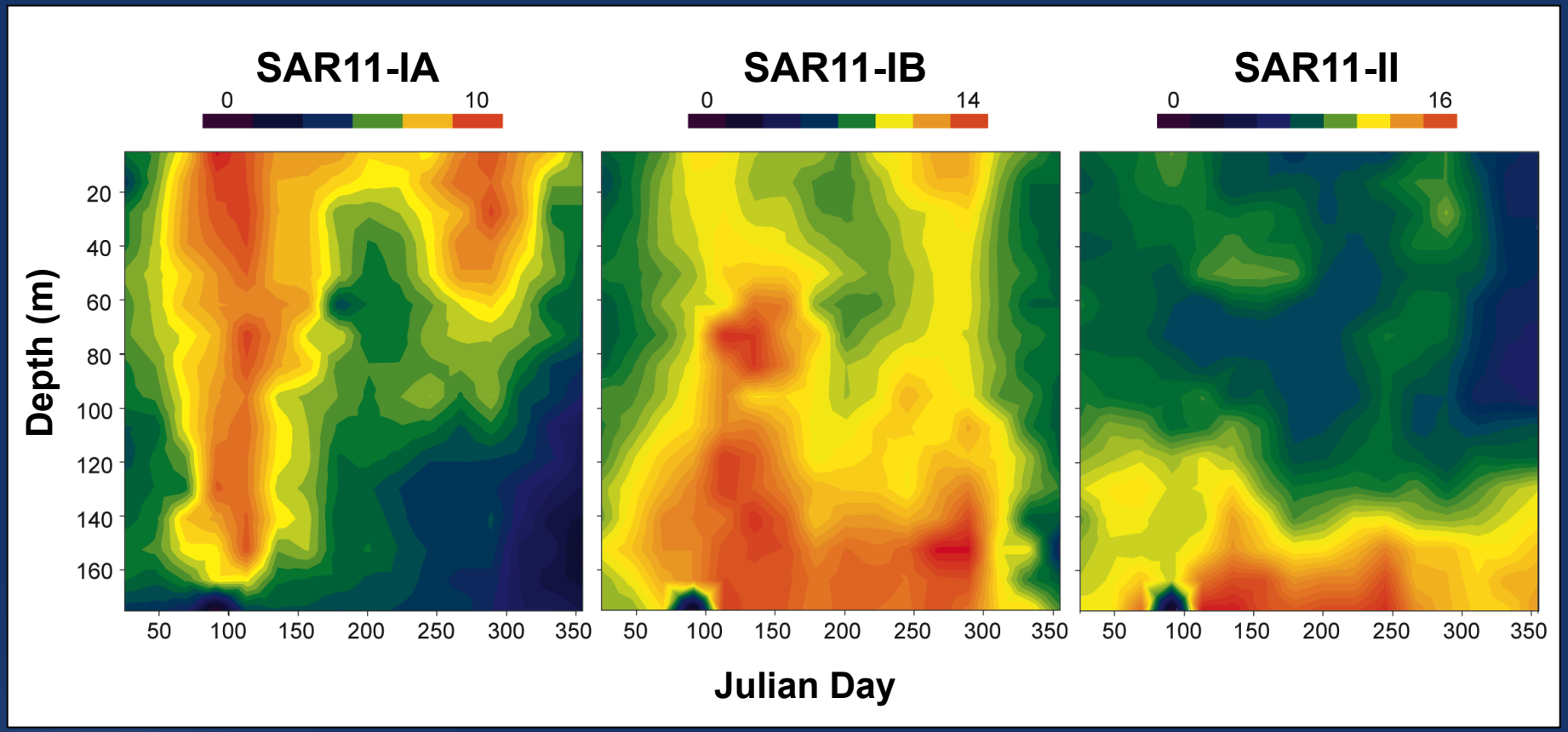
Depth specific distribution of SAR11 subgroups, Station ALOHA



How many SAR11 ecotypes are there?