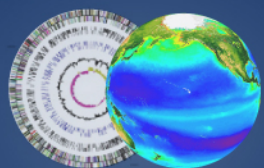


“High throughput” cultivation and strain HIMB100

Michael S. Rappé

June 9, 2010
Agouron Summer Course

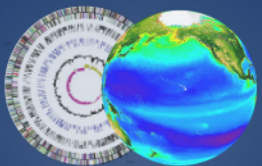
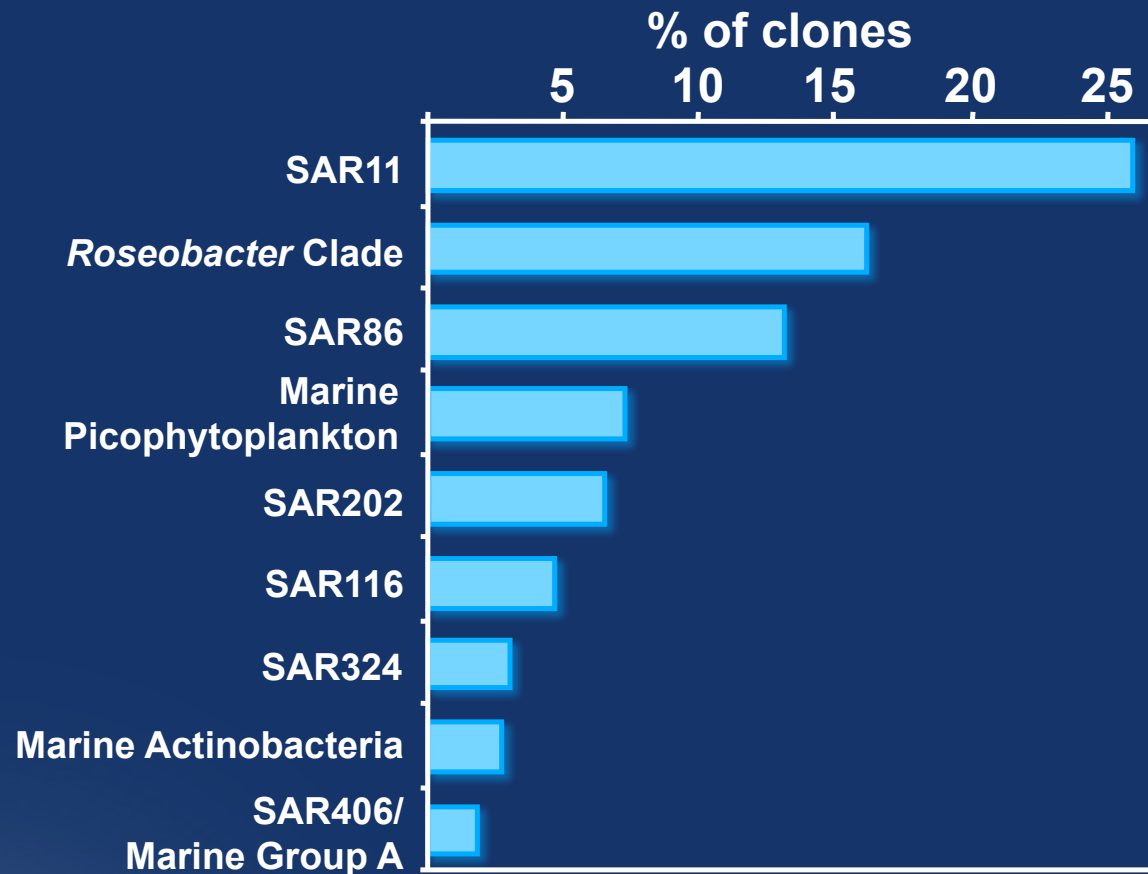


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Relative abundance of the major bacterial rRNA gene clone groups in seawater

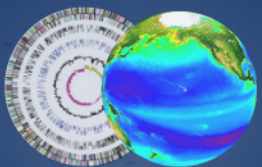
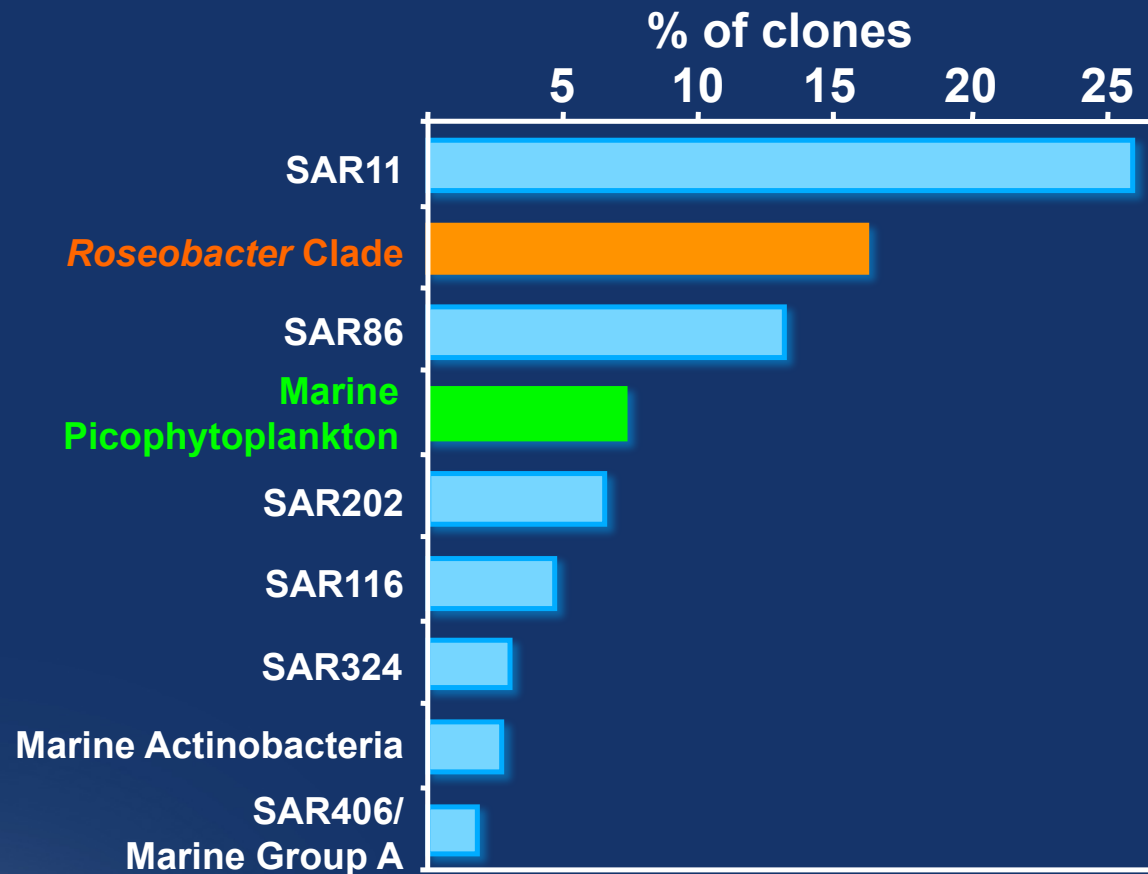


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Giovannoni & Rappé 2000

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



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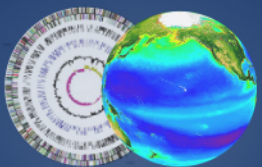
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Giovannoni & Rappé 2000

Why have abundant marine bacterioplankton such as SAR11 evaded laboratory isolation?

Some hypotheses:

- Slow growth and low cell densities are not detected
- Interactions with other organisms are required
- Growth only occurs in narrowly defined conditions that are not likely to be created by chance experimentation
- Trace contaminants in laboratory reagents are toxic



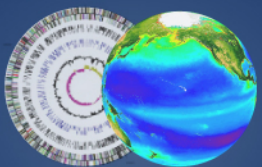
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Strategy for isolating new marine bacteria

- Reproduce the dilute conditions of natural systems
- Miniaturize the culturing process
- Decrease the limit of detection for cellular growth
- Identify isolates via rapid, high throughput methods (*in situ* hybridization, sequencing)
- Incorporate robotics/automation to increase the number of inoculated culture vessels and increase the diversity of culture conditions

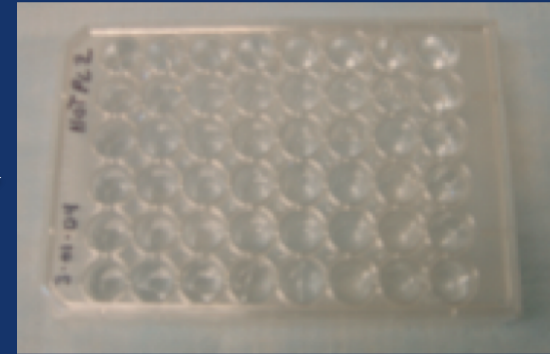
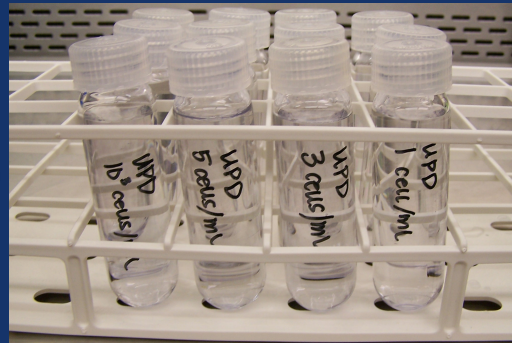
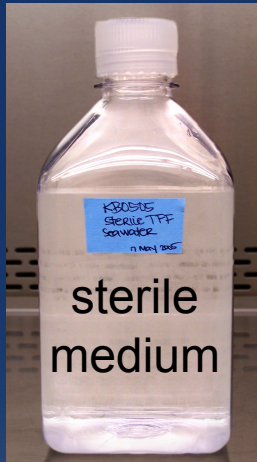
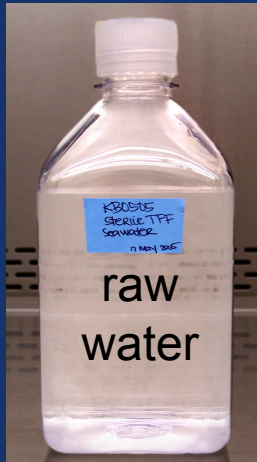


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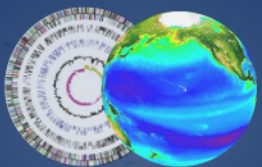
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“High-throughput cultivation”



Dilute cells of raw inoculum to desired concentration in sterile media

Array diluted samples in culture vessels and incubate

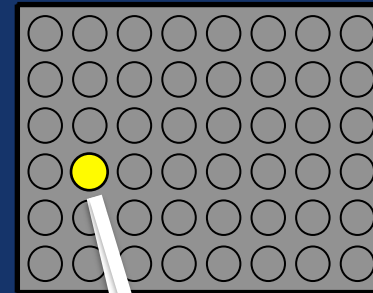
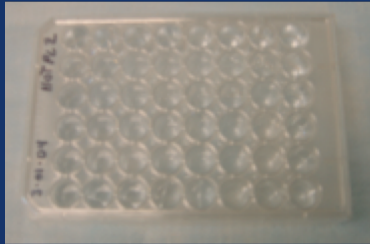


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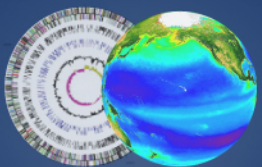
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“High-throughput cultivation”



DAPI stain and
with
cent beads

Array incubated
samples onto
polycarbonate
membrane



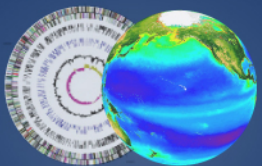
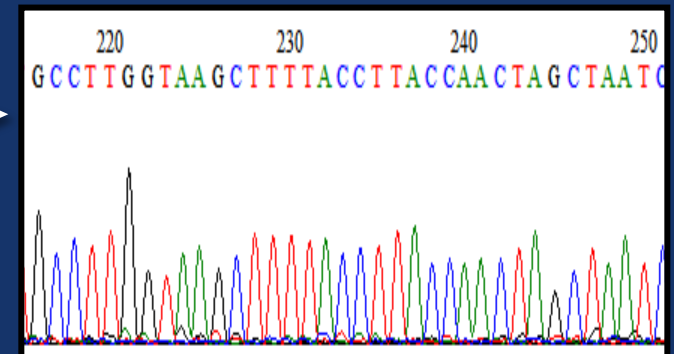
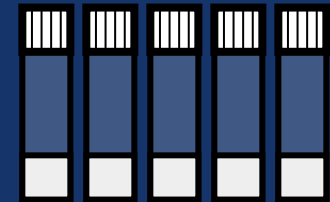
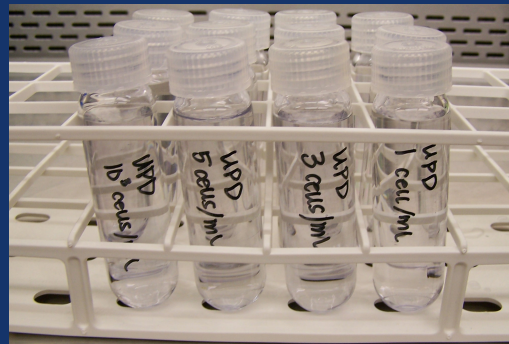
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“High-throughput cultivation”

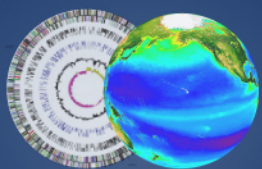
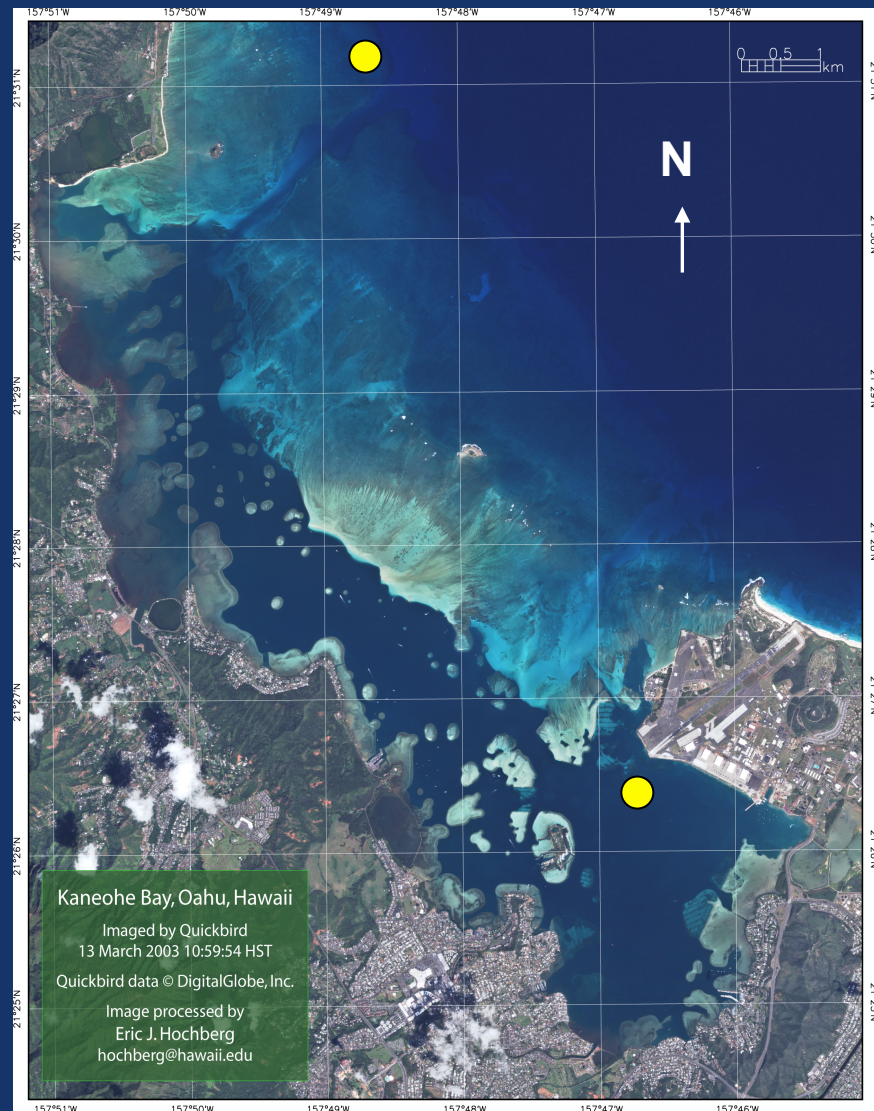
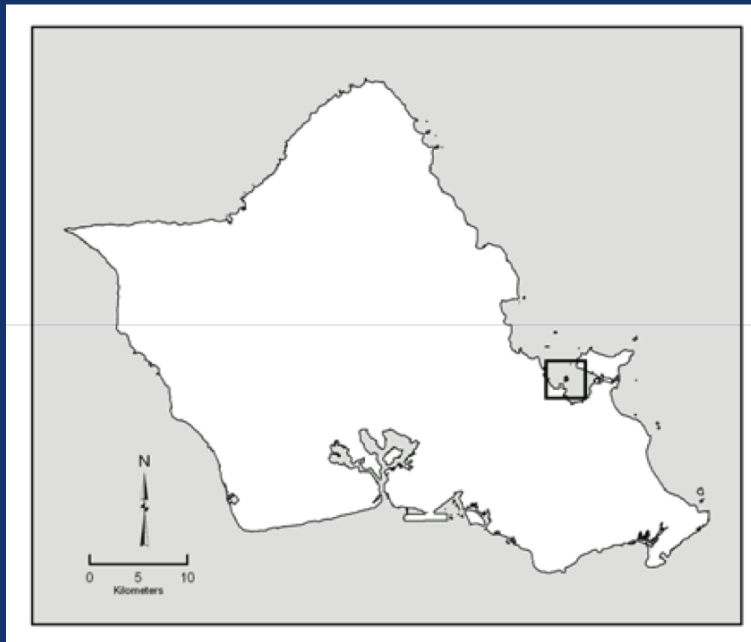
	1	2	3	4	5	6	7	8
A	○	○	●	○	○	●	○	○
B	○	○	○	○	○	●	○	○
C	○	●	○	●	○	○	○	○
D	○	○	●	○	○	○	○	○
E	○	○	○	○	○	○	●	○
F	○	●	○	●	○	○	○	○



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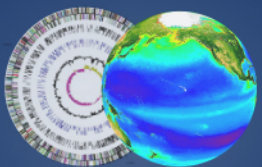
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Experimental design

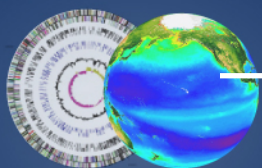
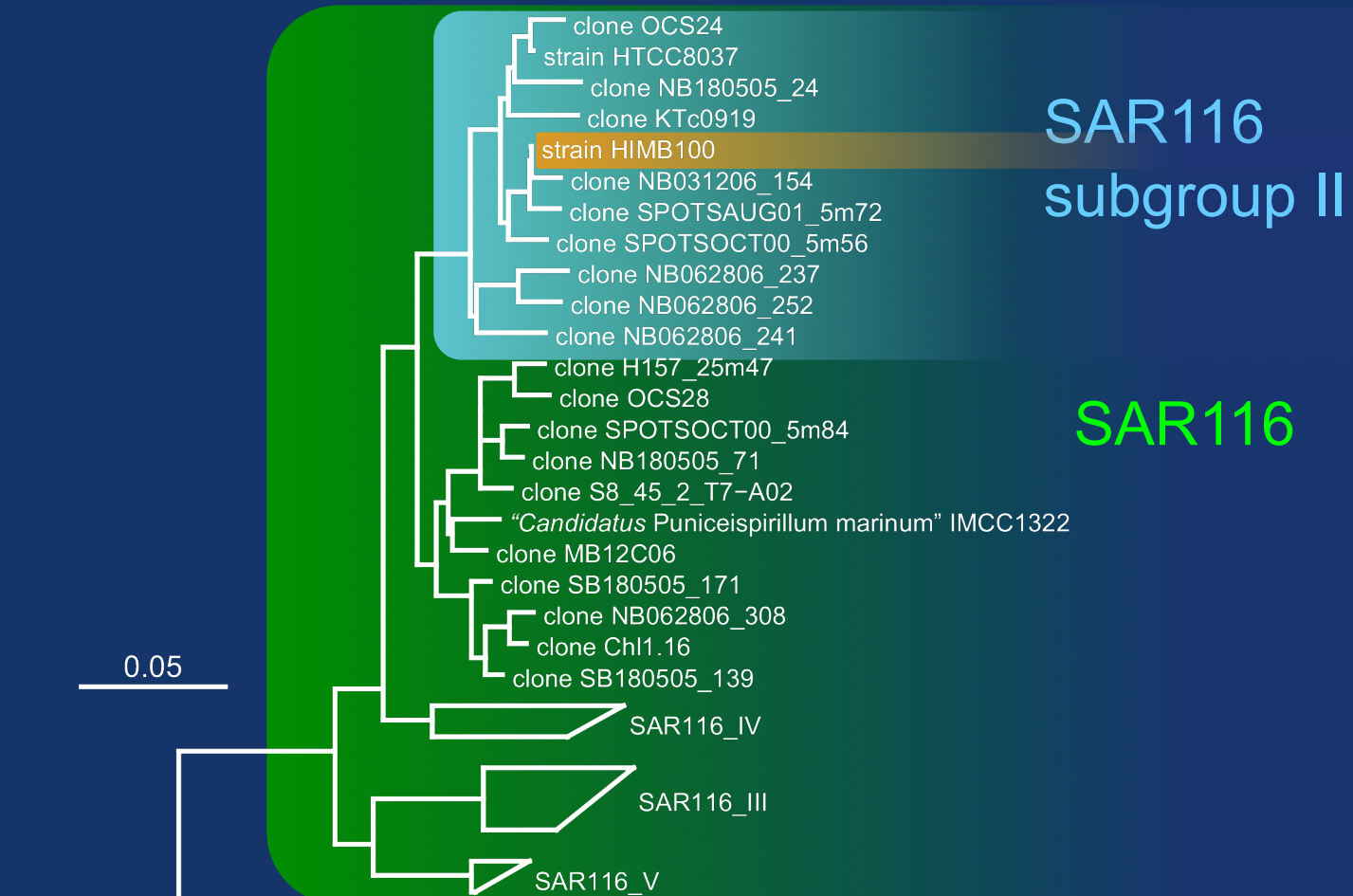
- **Sample collection:** May 17 (media) and 18 (inocula), 2005, Kaneohe Bay, Hawaii in the Pacific Ocean, surface water
- **Media:** sterile seawater with the following amendments:
 1. 1.0 μM ammonia, 1.0 μM nitrate, 0.1 μM phosphate
 2. (1) + 0.001% mixed carbon
 3. 0.1 μM urea, 0.1 μM phosphate, 0.001% DMSP
- **Inoculation:** 2 locations; 1-, 3- and 5-cell dilutions
- **Incubation:** 27C, 12:12 L/D cycle, screened after 3, 5, and 7 weeks



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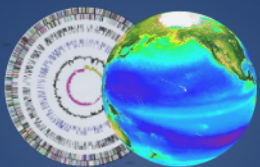
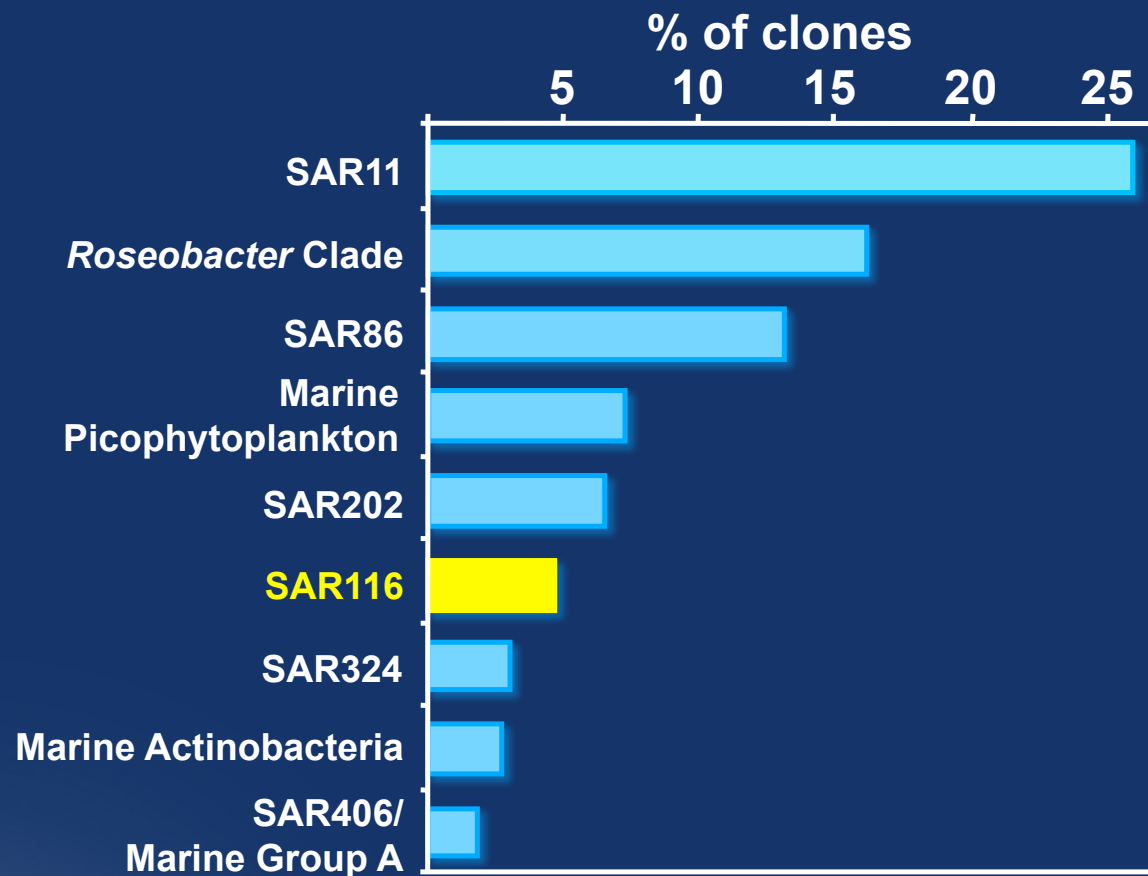


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Relative abundance of the major bacterial rRNA gene clone groups in seawater

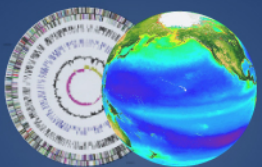


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Giovannoni & Rappé 2000

Affiliation	Strains recovered from:		Variation (bp)
	SB	NB	
Alphaproteobacteria			
SAR11 subclade IA	10	10	20
SAR11 subclade III	2	4	1
SAR11 subclade IIB	1	0	-
SAR116 subclade II	3	1	0
Aegean_169 clade	6	10	7
<i>Roseobacter</i> clade	5	17	4
Betaproteobacteria			
OM43 clade	4	7	1
Gammaproteobacteria			
OM60 clade	0	7	8
OM252 clade	5	3	0
Cyanobacteria			
<i>Synechococcus</i> clade II	8	11	2
<i>Synechococcus</i> clade X	2	2	2
Mixed cultures	8	13	

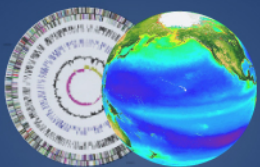
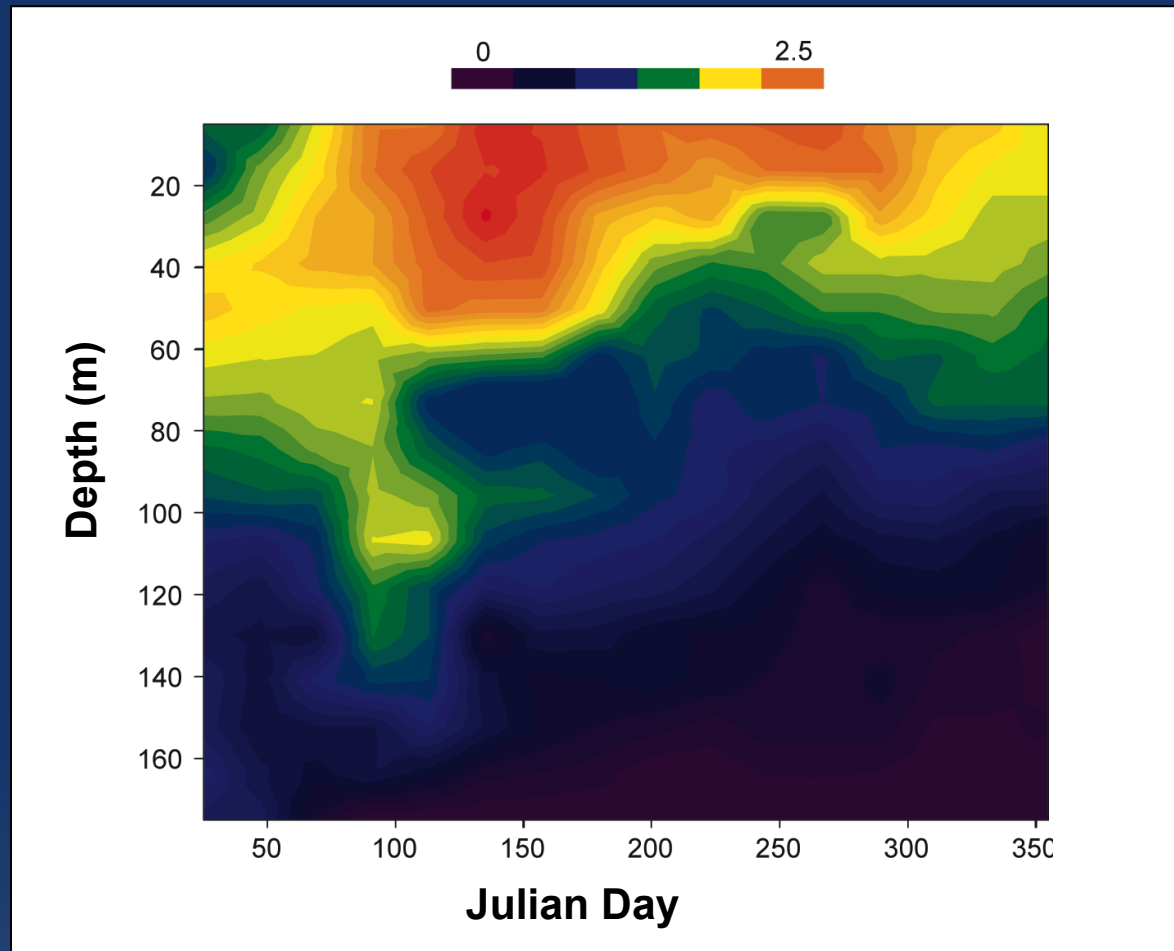


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Depth specific distribution of the SAR116 clade at Station ALOHA (T-RFLP)

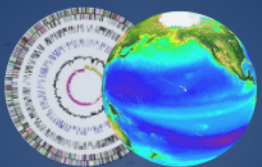
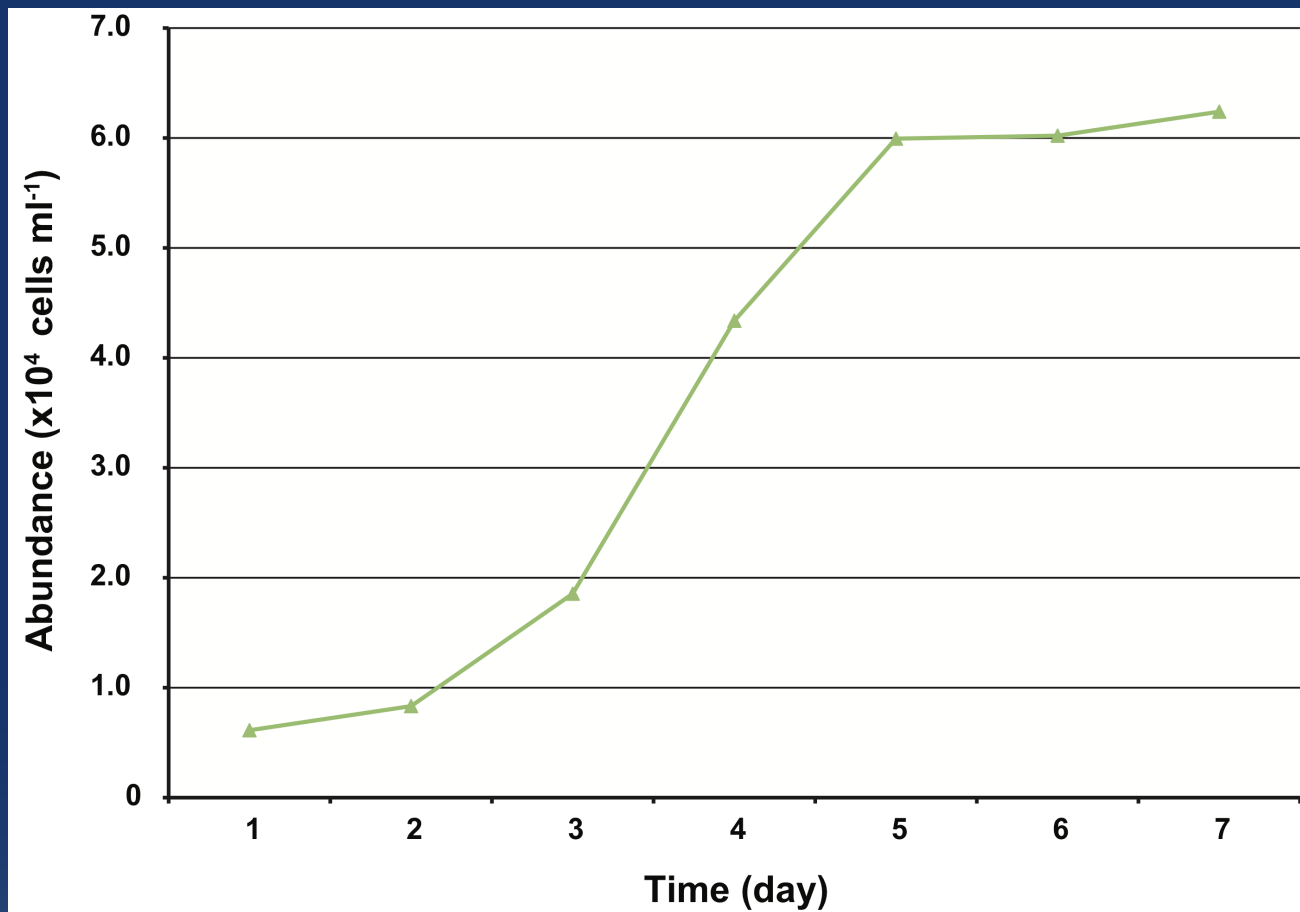


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Darin Hayakawa

Growth of str. HIMB100 in natural seawater media

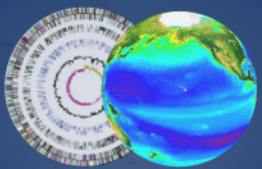


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Jana Grote

Culture scale-up

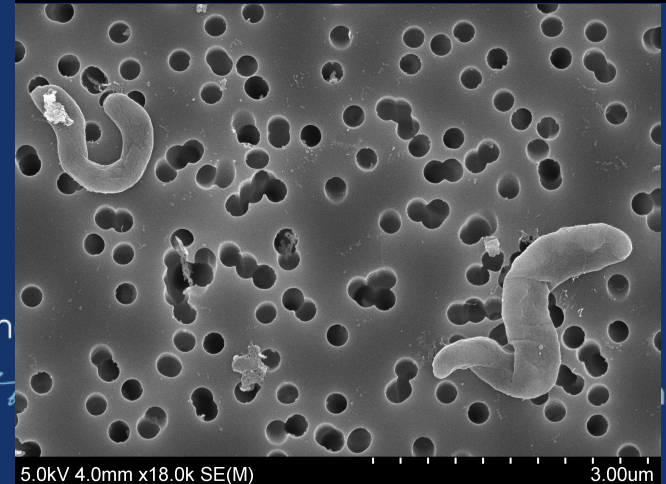
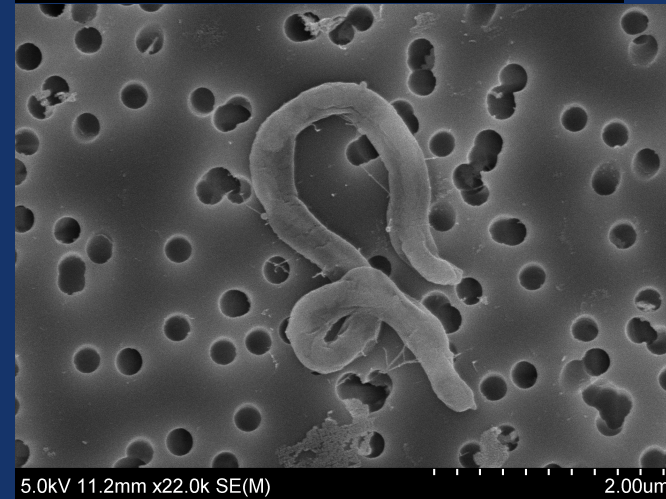
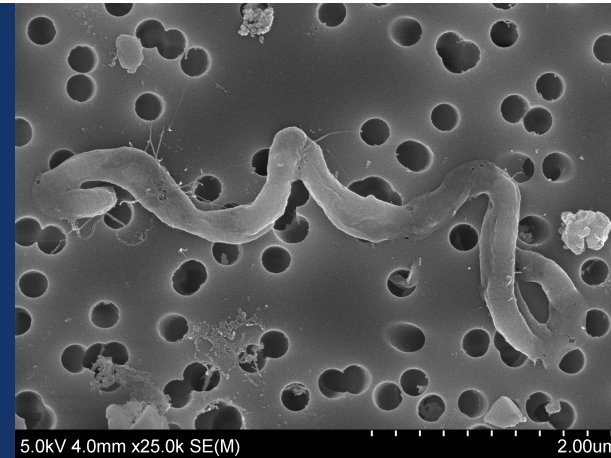


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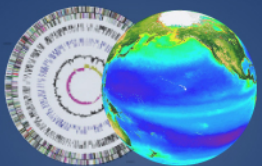
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SAR116



Jana Grote

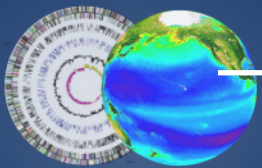
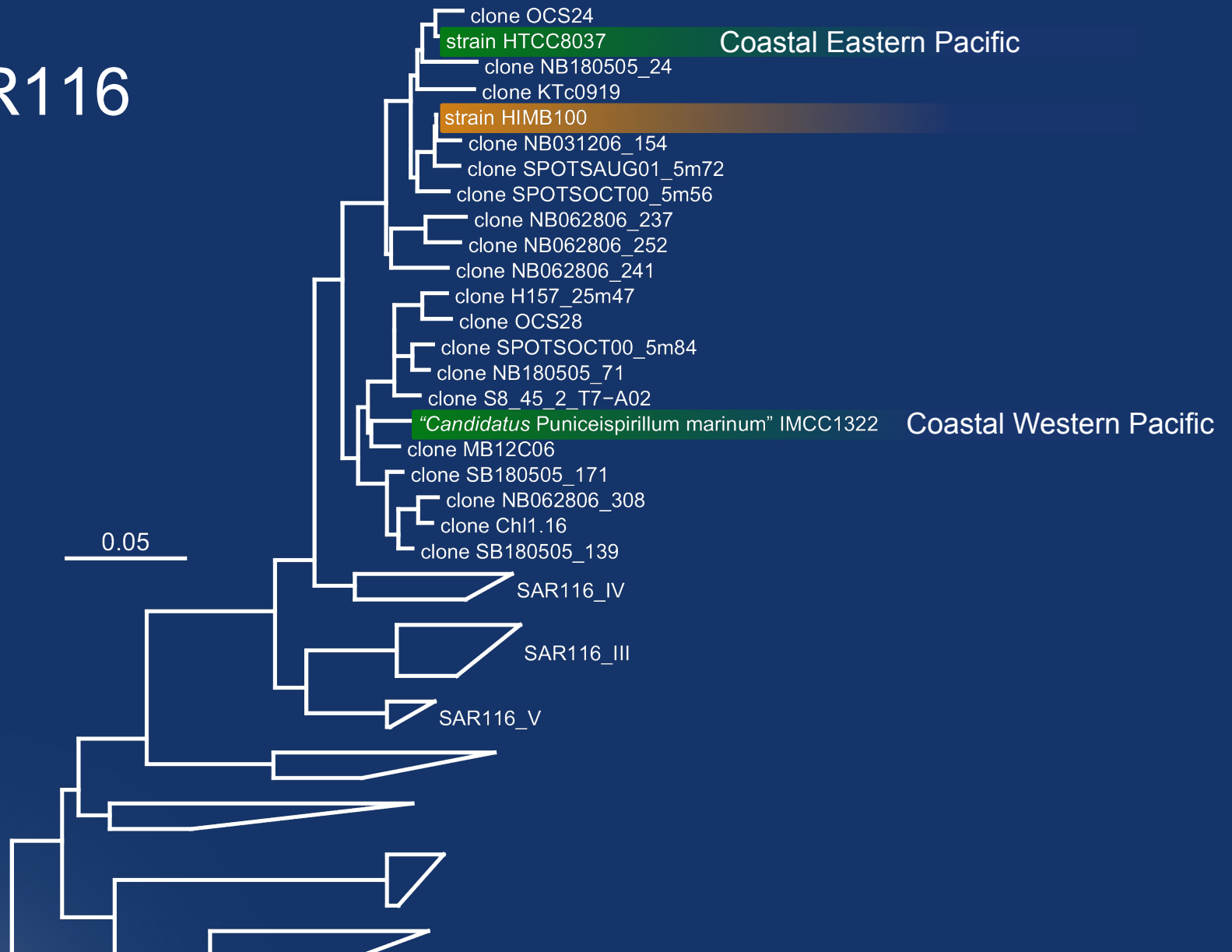


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SAR116



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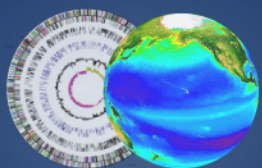
GENOME ANNOUNCEMENT

Complete Genome Sequence of “*Candidatus Puniceispirillum marinum*” IMCC1322, a Representative of the SAR116 Clade in the *Alphaproteobacteria*

Hyun-Myung Oh,^{1†} Kae Kyoung Kwon,^{2†} Innam Kang,¹ Sung Gyun Kang,²
Jung-Hyun Lee,² Sang-Jin Kim,^{2*} and Jang-Cheon Cho^{1*}

*Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Republic of Korea, 1 and
Marine Biotechnology Research Center, Korea Ocean Research & Development Institute,
Ansan 425-600, Republic of Korea 2*

The complete genome sequence of “*Candidatus Puniceispirillum marinum*” IMCC1322, the first cultured representative of the SAR116 clade in the *Alphaproteobacteria*, is reported here. The genome contains genes for proteorhodopsin, aerobic-type carbon monoxide dehydrogenase, dimethylsulfoniopropionate demethylase, and C₁ compound metabolism. The genome information proposes the SAR116 group to be metabolic generalists in ocean nutrient cycling.

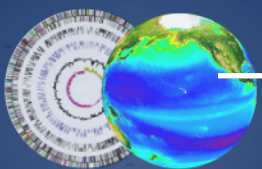
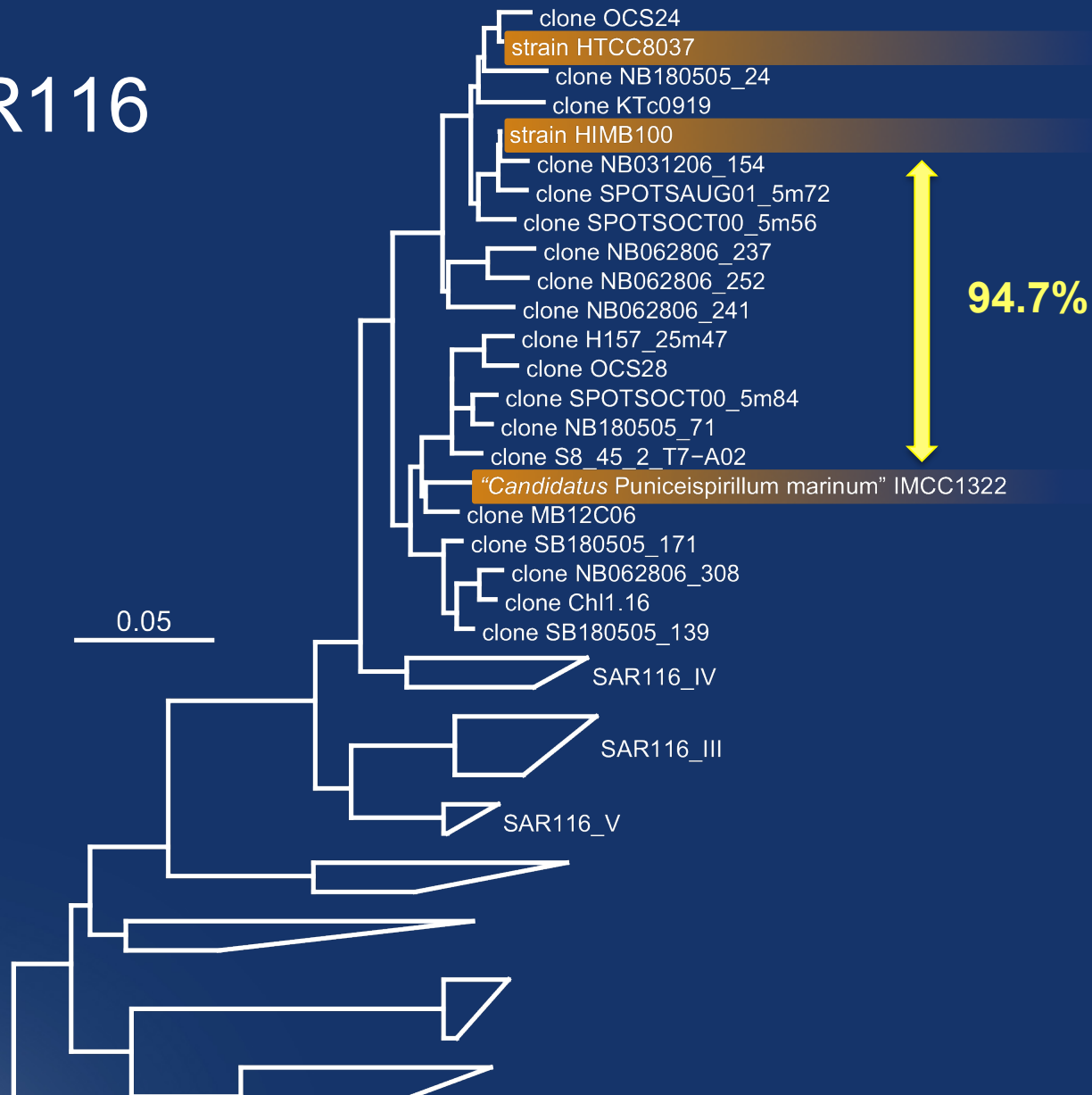


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SAR116



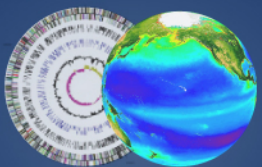
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“*Candidatus Puniceispirillum marinum*”

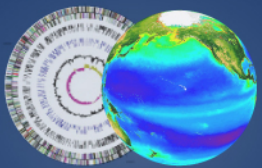
- 2.75 Mbp genome, 48.9% G+C
- 2,546 predicted ORFs
- Characteristics of note:
 - proteorhodopsin
 - carbon monoxide dehydrogenase
 - C₁ compound metabolism
 - dimethylsulfoniopropionate (DMSP) demethylase



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