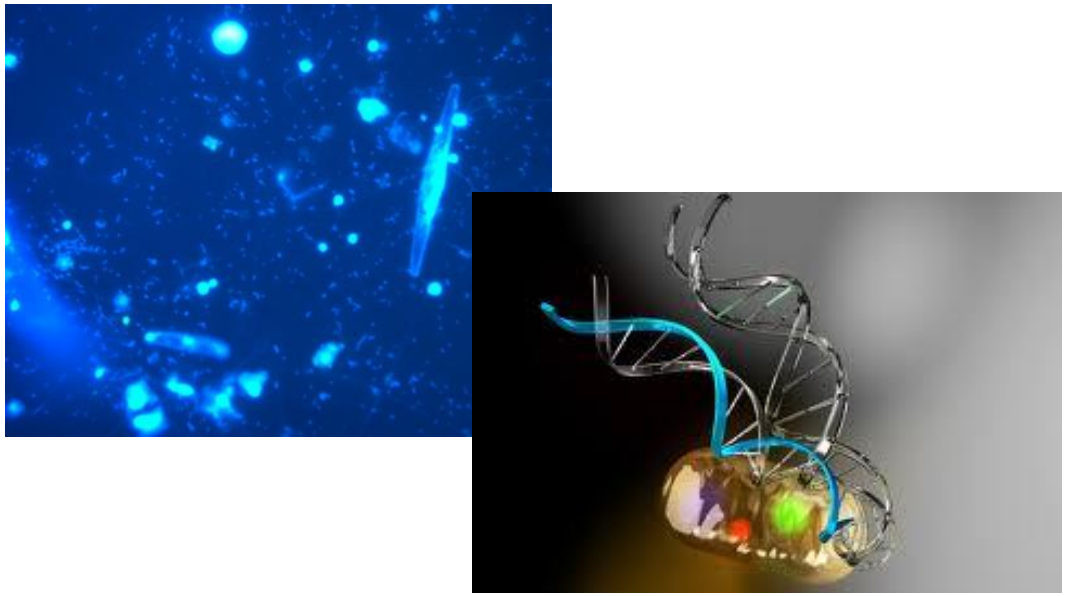
Single Cell Genomics



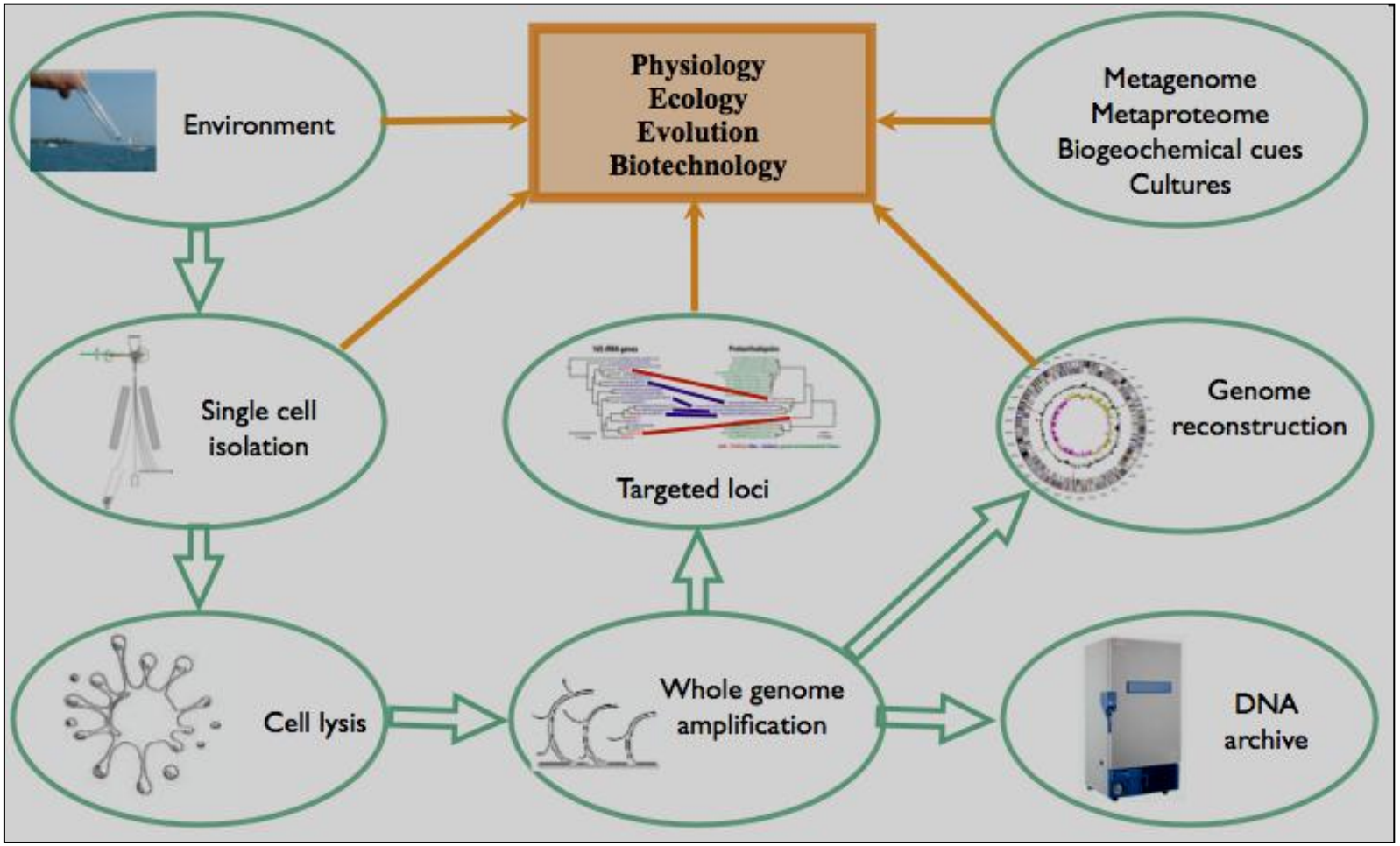
Manuel Martinez

A lesson by a few single cells

Manuel Martínez García

Universitat d'Alacant
Universidad de Alicante

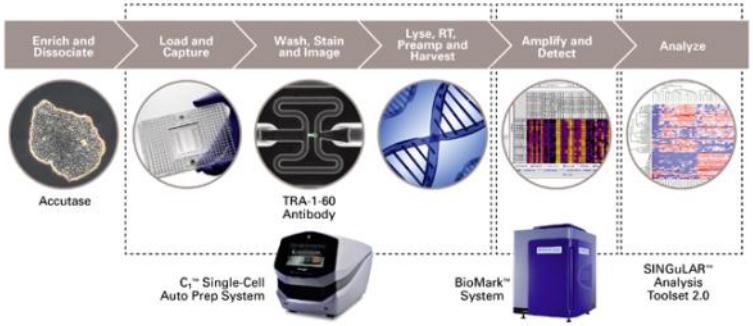
What is `Single Cell Genomics´



Genomic technologies are applied at the level of single cells, rather than at the level of an entire cell population

Single Cell Genomics in a few data

- A growing field since 2007
- >350 papers (>25 *Science/Nature/Cell/PNAS*)
- Microbiology, Genomics, Cancer, Biotechnology, Stem Cells, etc…



You can do it by yourself

Single Cell Genomics (SCG) Report 2013: Market Size, Segmentation, Growth, Competition and Trends

Like 0 8+1 More

DUBLIN, November 13, 2013 /PRNewswire/ --

RESEARCHANDMARKETS

Research and Markets (http://www.researchandmarkets.com/research/h74k64/single_cell) has announced the addition of the "Single Cell Genomics (SCG) Report 2013: Market Size, Segmentation, Growth, Competition and Trends" report to their offering.

(Logo: http://photos.prnewswire.com/prnh/20130307/600769)

More by this Source

Global and China Vitamin E Industry Report 2013
29 Nov, 2013, 14:49 GMT

Applying single cell technologies to…

LETTER

doi:10.1038/nature09807

Tumour evolution inferred by single-cell sequencing

Cancer

Nicholas Navin[1,2], Jude Kendall[1], Jennifer Troge[1], Peter Andrews[1], Linda Rodgers[1], Jeanne McIndoo[1], Kerry Cook[1], Asya Stepansky[1], Dan Levy[1], Diane Esposito[1], Lakshmi Muthuswamy[3], Alex Krasnitz[1], W. Richard McCombie[1], James Hicks[1] & Michael Wigler[1]

Potential for Chemolithoautotrophy Among Ubiquitous Bacteria Lineages in the Dark Ocean

Microbial Ecology

Brandon K. Swan,[1] Manuel Martinez-Garcia,[1] Christina M. Preston,[2] Alexander Sczyrba,[3] Tanja Woyke,[3] Dominique Lamy,[4]* Thomas Reinthaler,[4] Nicole J. Poulton,[1] E. Dashiell P. Masland,[1] Monica Lluesma Gomez,[1] Michael E. Sieracki,[1] Edward F. DeLong,[5] Gerhard J. Herndl,[4] Ramunas Stepanauskas[1]†

Science 28 January 2011:
Vol. 331 no. 6016 pp. 463-467
DOI: 10.1126/science.1200387

‹ Prev | Table of Contents | Next ›

REPORT

Metagenomic Discovery of Biomass-Degrading Genes and Genomes from Cow Rumen

Matthias Hess[1,2,†,*], Alexander Sczyrba[1,2,†,*], Rob Egan[1,2], Tae-Wan Kim[3], Harshal Chokhawala[3], Gary Schroth[4], Shujun Luo[4], Douglas S. Clark[3,5], Feng Chen[1,2], Tao Zhang[1,2], Roderick I. Mackie[6], Len A. Pennacchio[1,2], Susannah G. Tringe[1,2], Axel Visel[1,2], Tanja Woyke[1,2], Zhong Wang[1,2], and Edward M. Rubin[1,2,‡]

Biotechnology and many more topics

NATURE METHODS | REVIEW

Development and applications of single-cell transcriptome analysis

Fuchou Tang, Kaiqin Lao & M Azim Surani

Affiliations | Corresponding authors

Nature Methods (2011) | doi:10.1038/nmeth.1557
Published online 30 March 2011

Novel metabolic features found in the SAG data set.

nature International weekly journal of science

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Archive > Volume 499 > Issue 7459 > Articles > Article

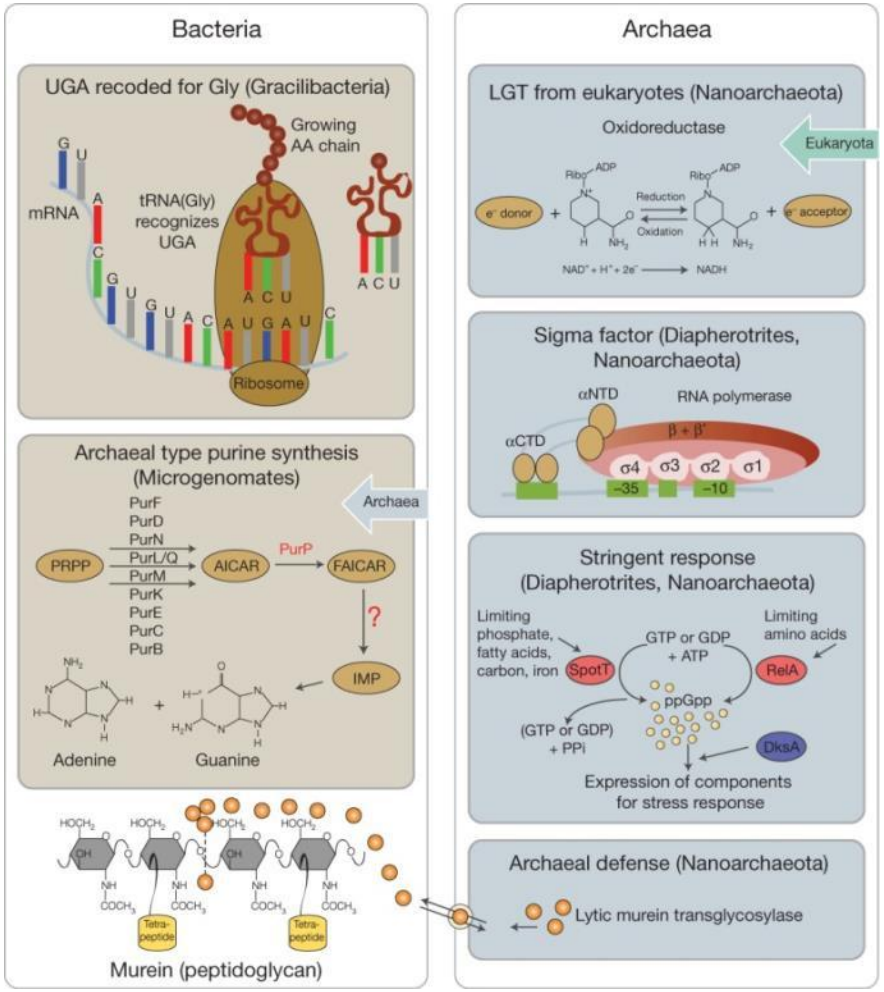
NATURE | ARTICLE OPEN

日本語要約

Insights into the phylogeny and coding potential of microbial dark matter

Christian Rinke, Patrick Schwientek, Alexander Sczyrba, Natalia N. Ivanova, Iain J. Anderson, Jan-Fang Cheng, Aaron Darling, Stephanie Malfatti, Brandon K. Swan, Esther A. Gies, Jeremy A. Dodsworth, Brian P. Hedlund, George Tsiamis, Stefan M. Sievert, Wen-Tso Liu, Jonathan A. Eisen, Steven J. Hallam, Nikos C. Kyrpides, Ramunas Stepanauskas, Edward M. Rubin, Philip Hugenholtz & Tanja Woyke

Affiliations | Contributions | Corresponding authors

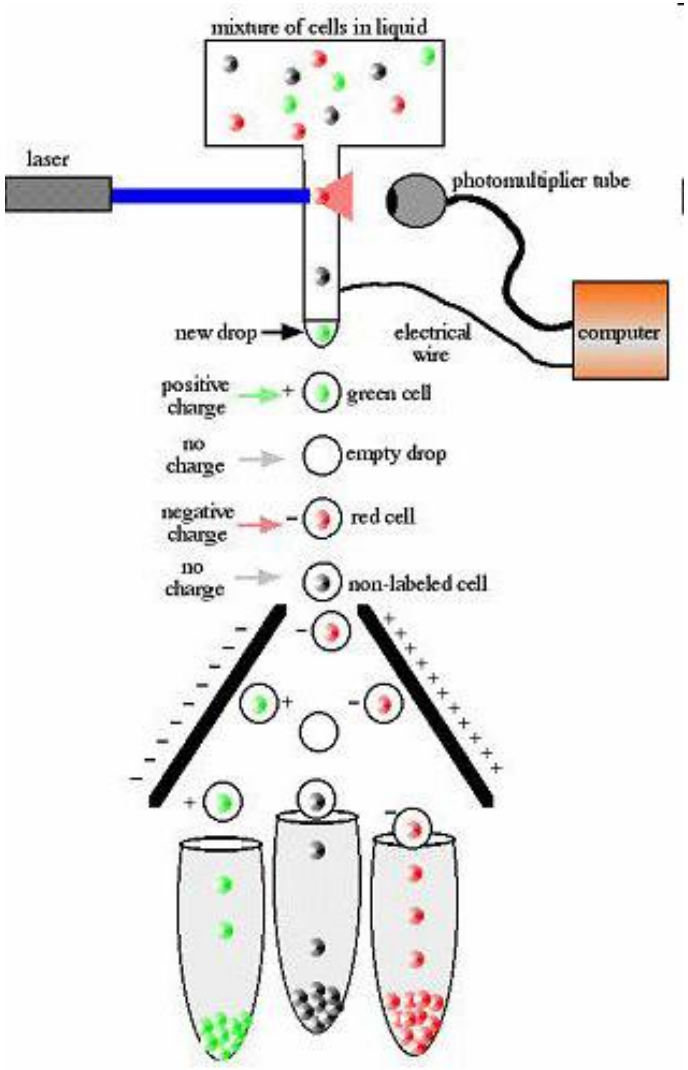


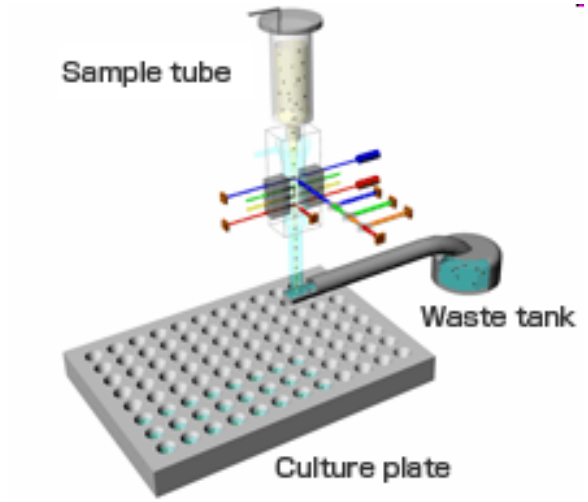
C Rinke *et al. Nature 2013*, 1-7 (2013) doi:10.1038/nature12352

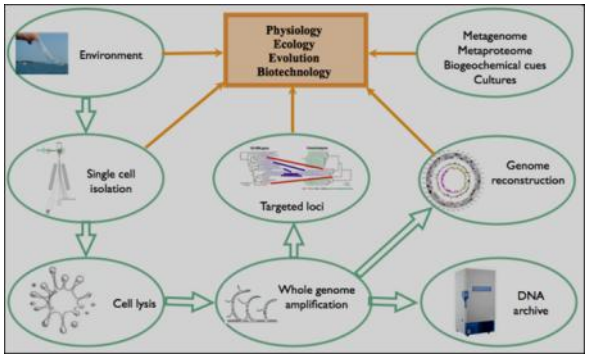
nature

1.“Everything starts with just a single cell”

Fluorescence Activated Cell Sorting vs Microfluidics



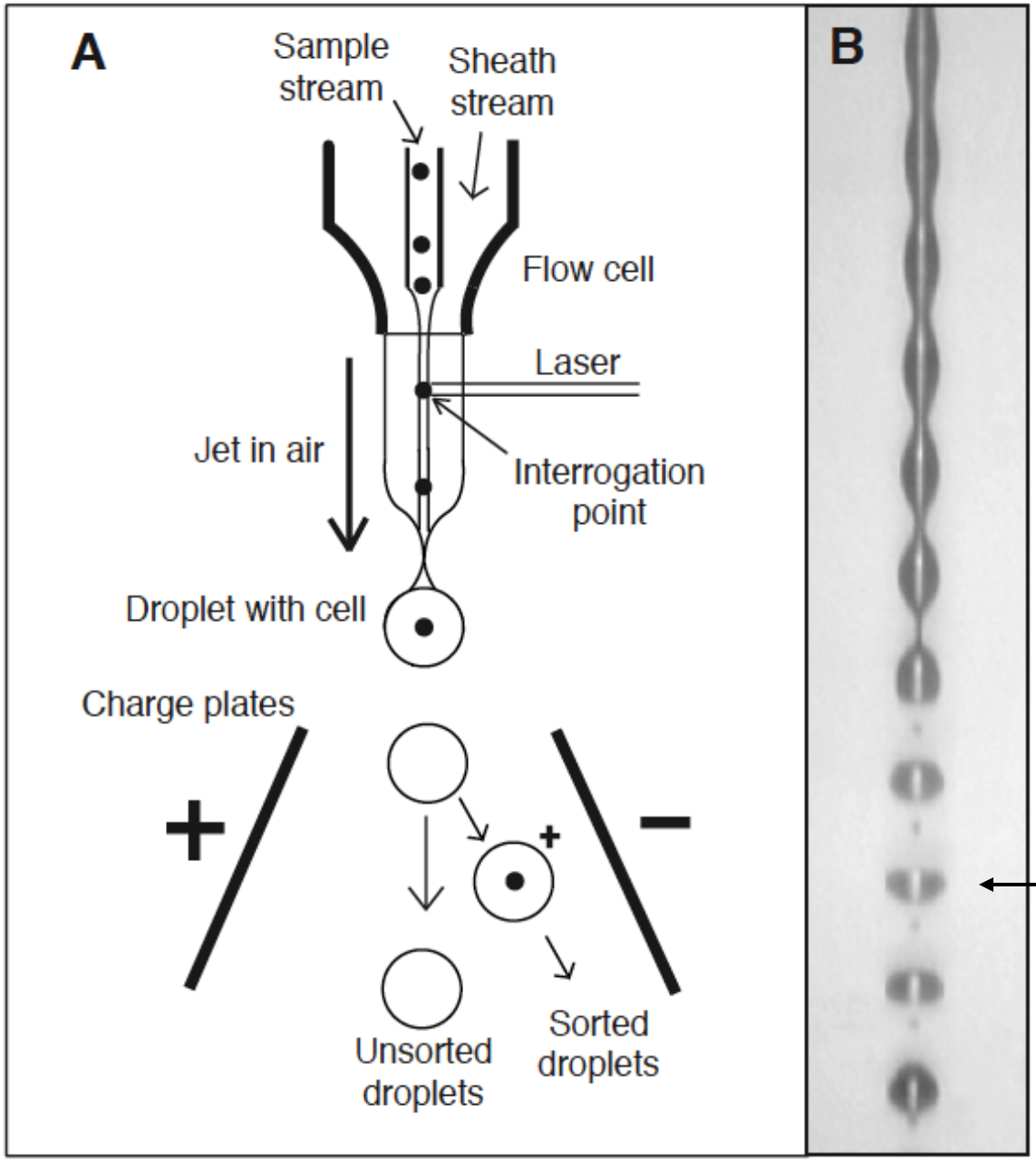




BIGELOW LABORATORY
Single Cell Genomics Center

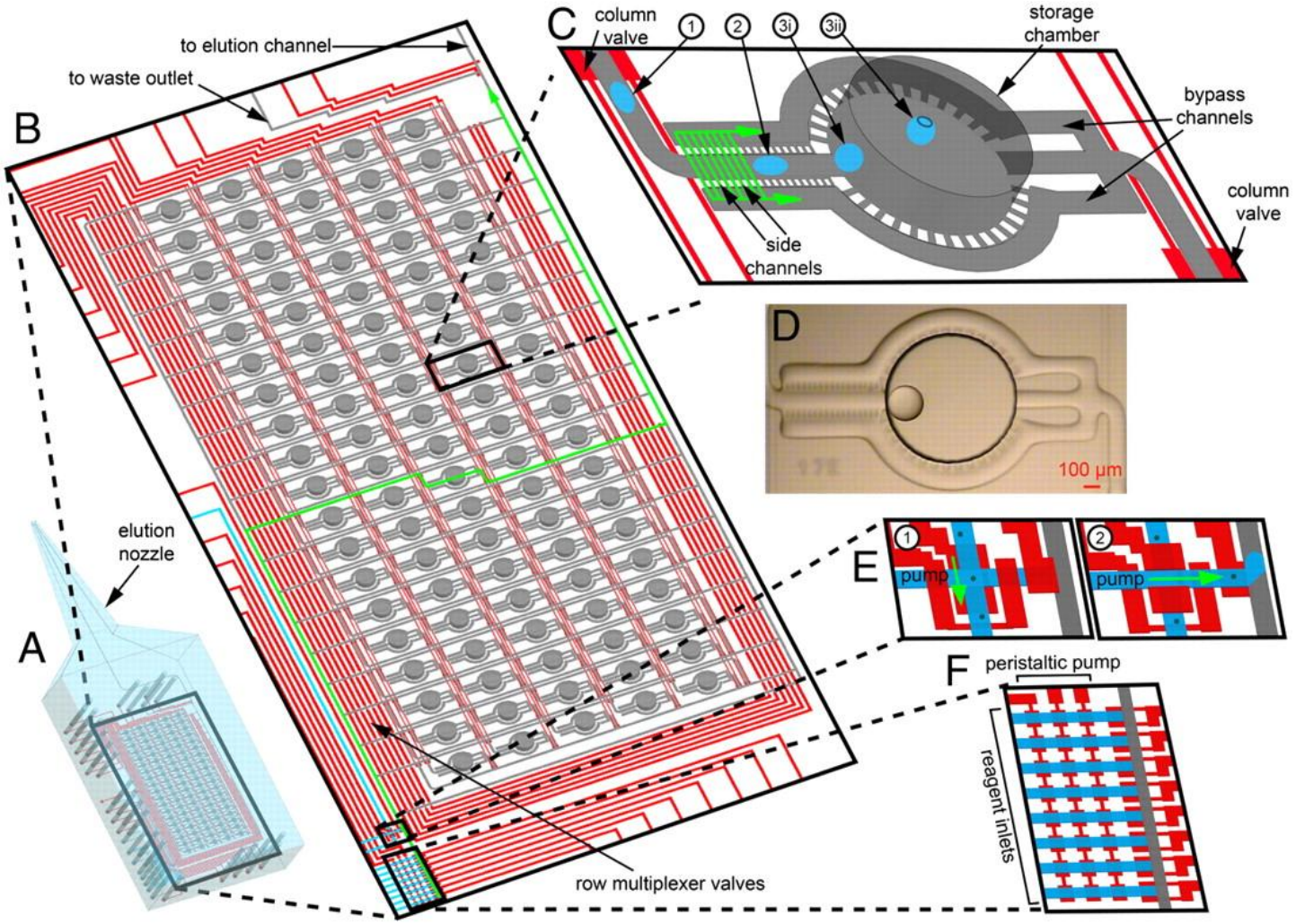
Cutting-edge services for basic research and biotechnology applications

Sorting


A
Sample stream
Sheath stream
Flow cell
Laser
Jet in air
Interrogation point
Droplet with cell
Charge plates
+
−
Unsorted droplets
Sorted droplets
B
The droplet

Programmable microfluidic reaction array.

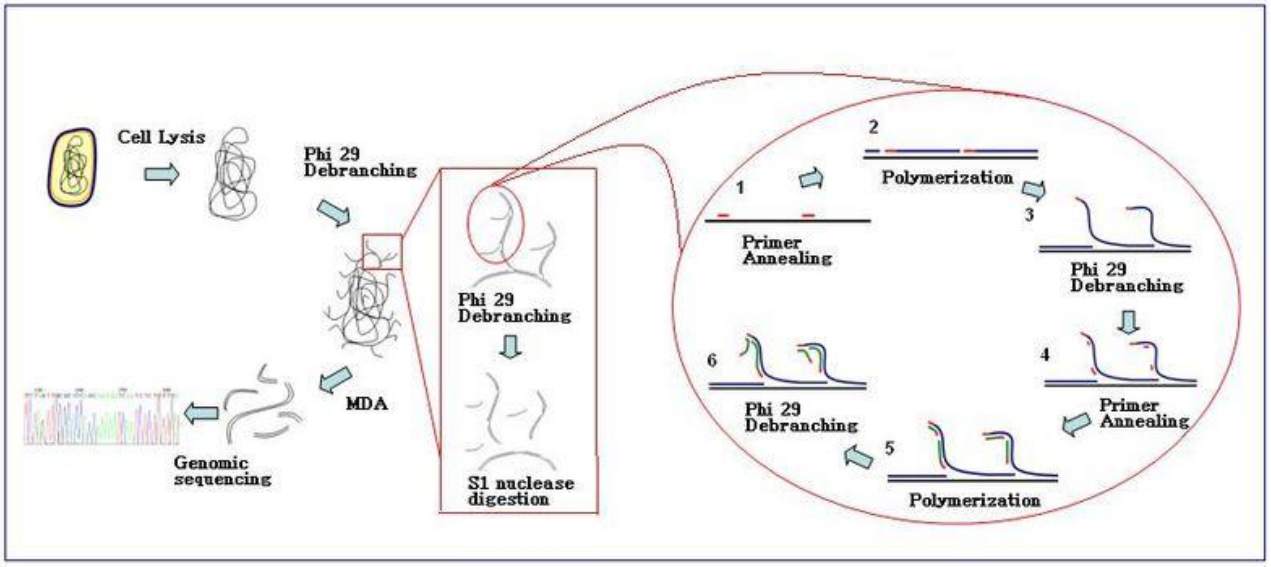
Available online at www.sciencedirect.com
SciVerse ScienceDirect
Current Opinion in Biotechnology
ELSEVIER
Microfluidics for single cell analysis
Huabing Yin[1] and Damian Marshall[2]



Leung K et al. PNAS 2012;109:7665-7670

©2012 by National Academy of Sciences

2. (So far), whole genome amplification is needed



nature biotechnology

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NATURE BIOTECHNOLOGY | RESEARCH | ARTICLE

Massively parallel polymerase cloning and genome sequencing of single cells using nanoliter microwells

Jeff Gole, Athurva Gore, Andrew Richards, Yu-Jui Chiu, Ho-Lim Fung, Diane Bushman, Hsin-I Chiang, Jerold Chun, Yu-Hwa Lo & Kun Zhang

Affiliations | Contributions | Corresponding author

Nature Biotechnology (2013) | doi:10.1038/nbt.2720
Received 23 April 2013 | Accepted 13 September 2013 | Published online 10 November 2013

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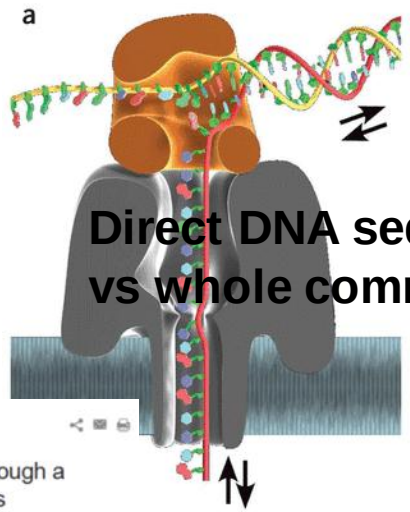
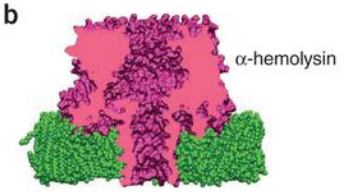
Abstract

Abstract • Introduction • Results • Discussion • Methods • Accession codes • References • Acknowledgments • Author information • Supplementary information

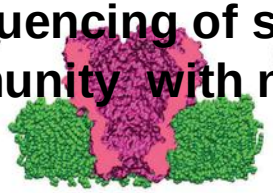
Genome sequencing of single cells has a variety of applications, including characterizing difficult-to-culture microorganisms and identifying somatic mutations in single cells from mammalian tissues. A major hurdle in this process is the bias in amplifying the genetic material from a single cell, a procedure known as polymerase cloning. Here we describe the microwell displacement amplification system (MIDAS), a massively parallel polymerase cloning method in which single cells are randomly distributed into hundreds to thousands of nanoliter wells and their genetic material is simultaneously amplified for shotgun sequencing. MIDAS reduces amplification bias

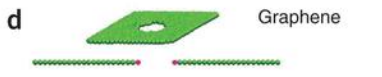
2 weeks ago: removing MDA bias





Direct DNA sequencing of single cells vs whole community with nanopores





NATURE NANOTECHNOLOGY | ARTICLE

Detecting the translocation of DNA through a nanopore using graphene nanoribbons

F. Traversi, C. Raillon, S. M. Benameur, K. Liu, S. Khlybov, M. Tosun, D. Krasnozhon, A. Kis & A. Radenovic

Affiliations | Contributions | Corresponding author

Nature Nanotechnology (2013) | doi:10.1038/nnano.2013.240
Received 31 October 2012 | Accepted 14 October 2013 | Published online 17 November 2013

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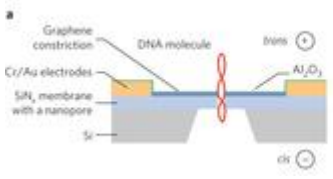
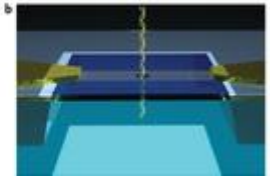
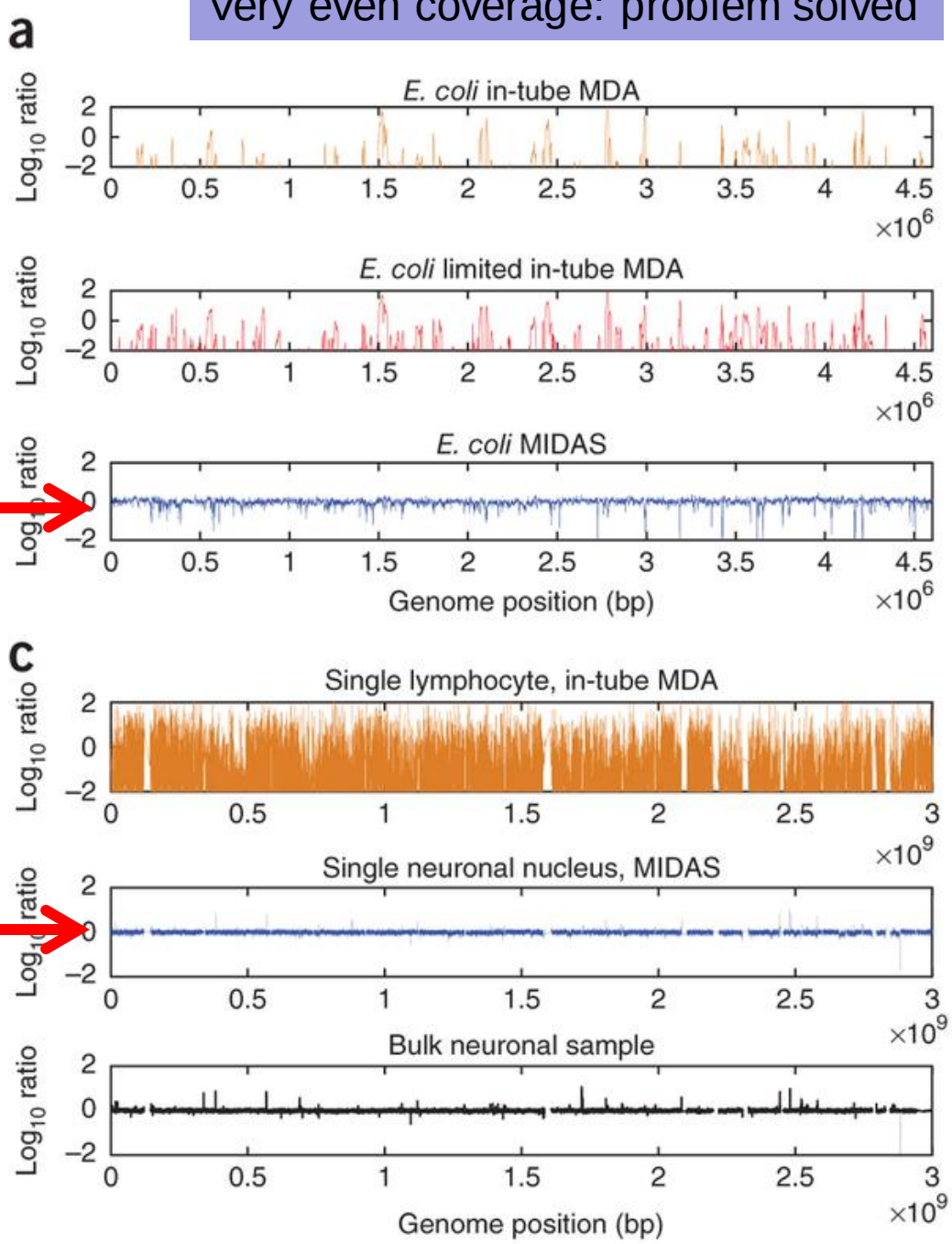




Very even coverage: problem solved



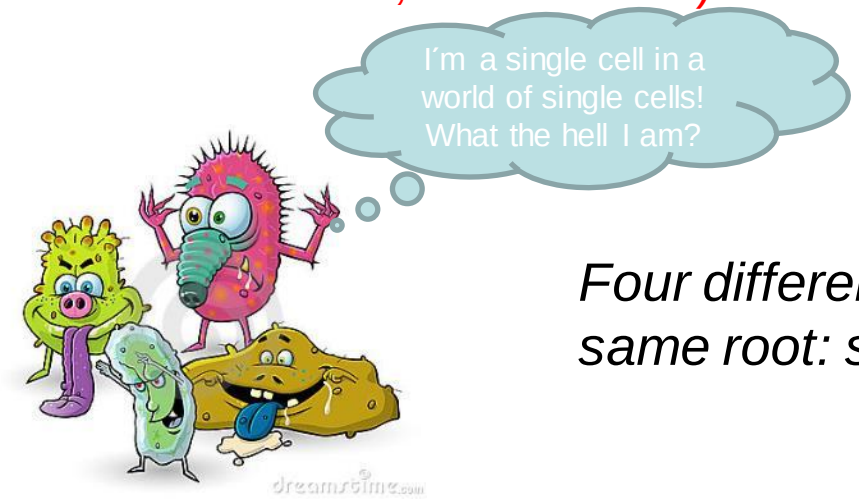
Doing Single Cell Sequencing… **we**:

1) Discovered widespread planktonic *Verrucomicrobia* as key active polysaccharide degraders in the *microbial loop (Martínez-Garcia et al., 2012, PLoS ONE)*

2) Identified predominant photoheterotrophs and chemoautotrophs in freshwater bacterioplankton (*Martínez-García et al., 2012, ISME J*)

3) Unveiled the ecological interaction between protist and bacteria in natural assemblages: predation and symbiosis (*Martínez-García et al., 2012, ISME J)*

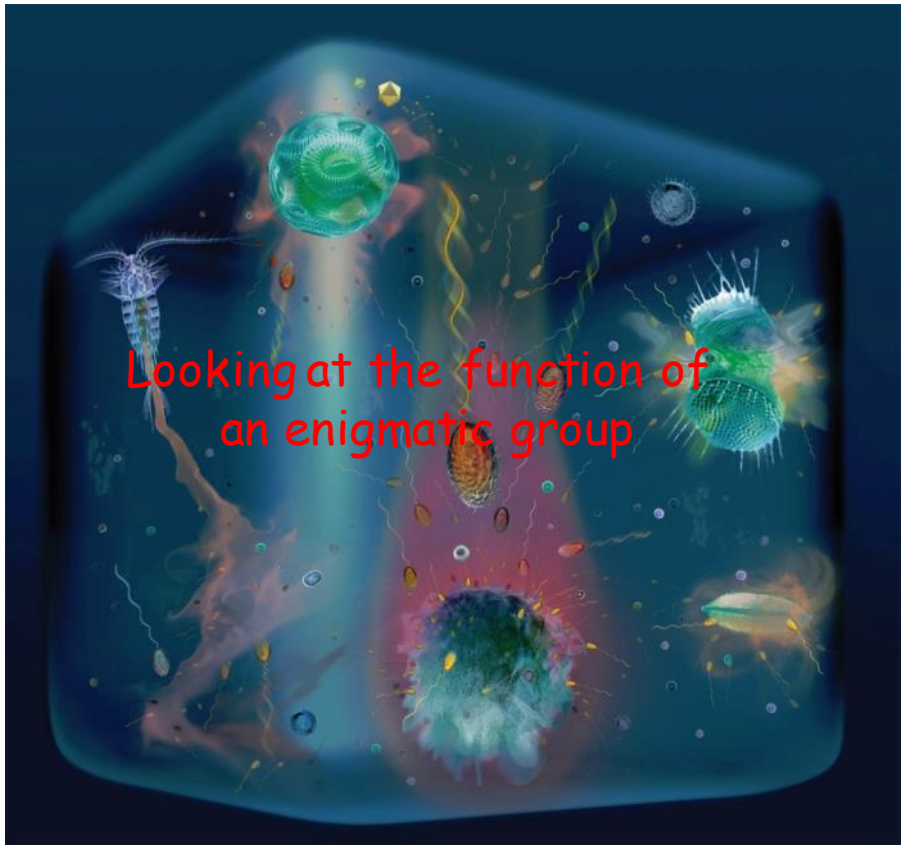
4) Assign virus to host in natural uncultured assemblages: one at a time (*Martínez-García and Santos et al., under review*)



Four different stories with the same root: single cell sequencing

PART 1

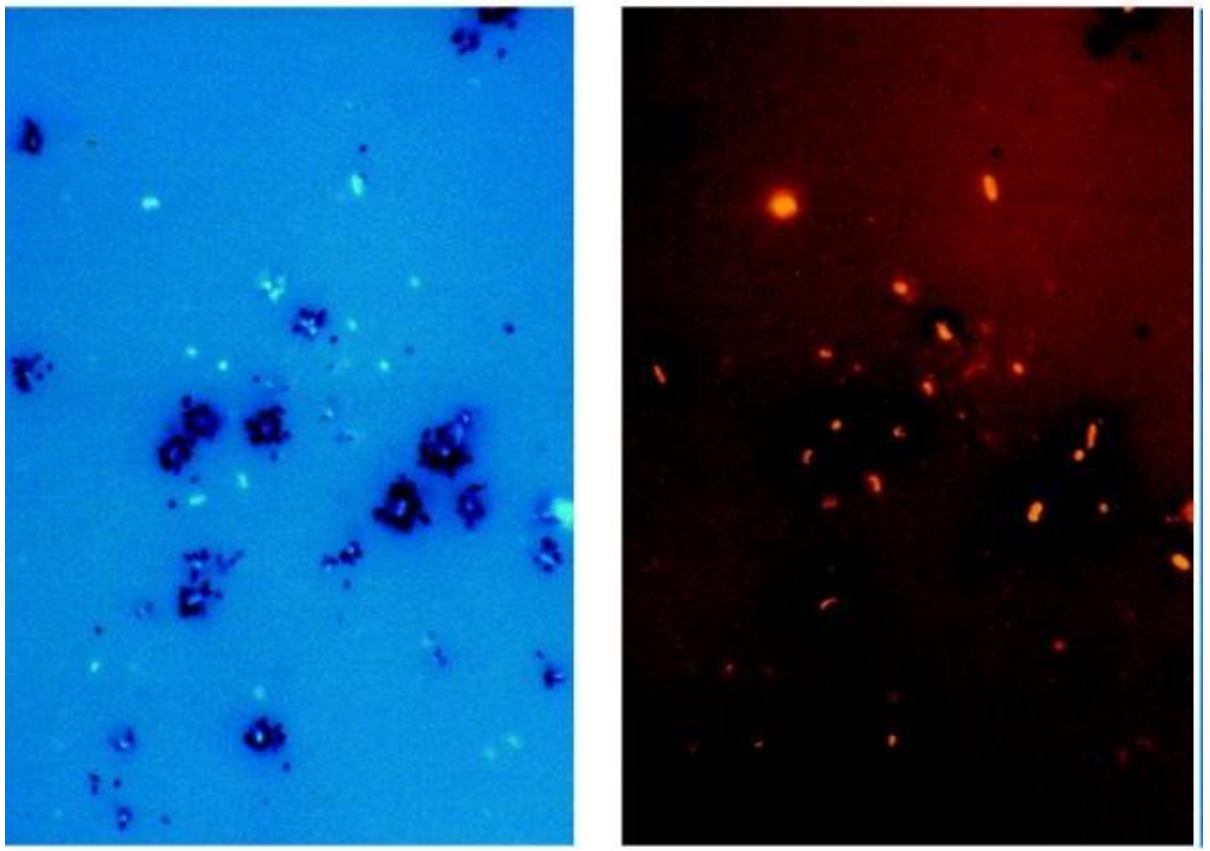
Single Cell Genomics suggests *Verrucomicrobia* as key polysaccharide degraders in planktonic ecosystems



1) Hydrolysis of polymers (polysaccharides, peptides and lipids) is a bottleneck in DOM mineralization in the microbial loop

2) Studies have focused in the identification of bacteria involved in labile-DOM remineralization (i.e. glucose and aa)

3) Two papers on the identification of bacteria involved HMW-DOM remineralization (chitin and EPS) by Microautoradiography+FISH ⟶ ***Bacteroidetes***



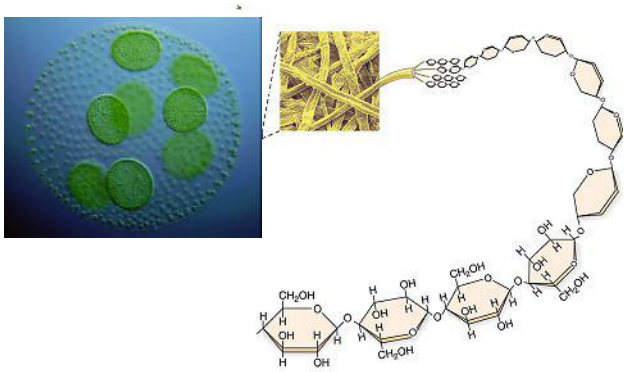
Cottrell and Kirchman, 2003

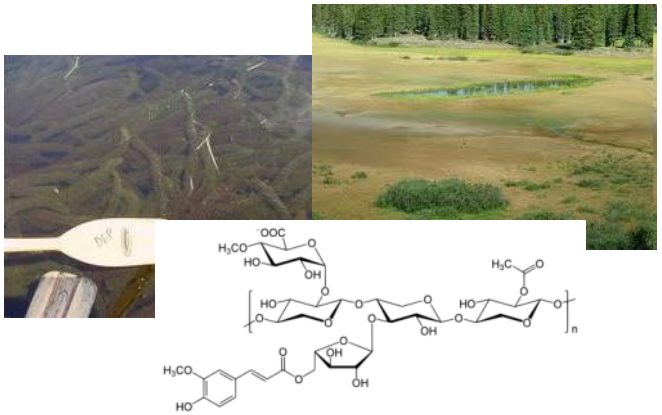
Relevance of polysaccharides in marine systems

15% of POC

32% of HMW-DOM

50% of total phytoplankton primary production





5-15 billion metric tons of the **LAMINARIN,** one of the most common storage polysaccharides, are produced annually by algae and phytoplankton

↓

Major polysaccharide in marine and freshwater systems

↘

XYLAN is a major polysaccharide hemicellulose produced by plants and algae (2nd most abundant glucan)

↓

Major polysaccharide in freshwater, soils, estuaries, and coastal areas

↙

It has been demonstrated the bacterial hydrolysis

Identify marine and freshwater bacterioplankton involved in the degradation of these two major polysaccharides in aquatic environments

☆ *Maine goal of the project*

Samples incubated with fluorescently labeled laminarin and xylan

polysaccharide

Fluorescent tag

Fluorescent tag

environment

Glycosyl hydrolase

Periplasm

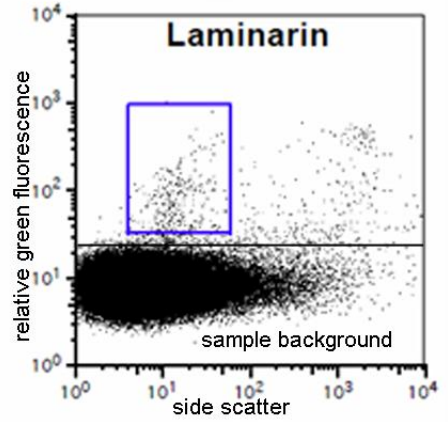
Inner membrane

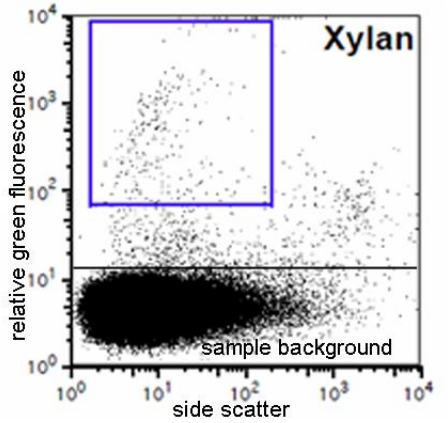
Cytoplasm

Oligosaccharide or disaccharide (max. 600 Da)

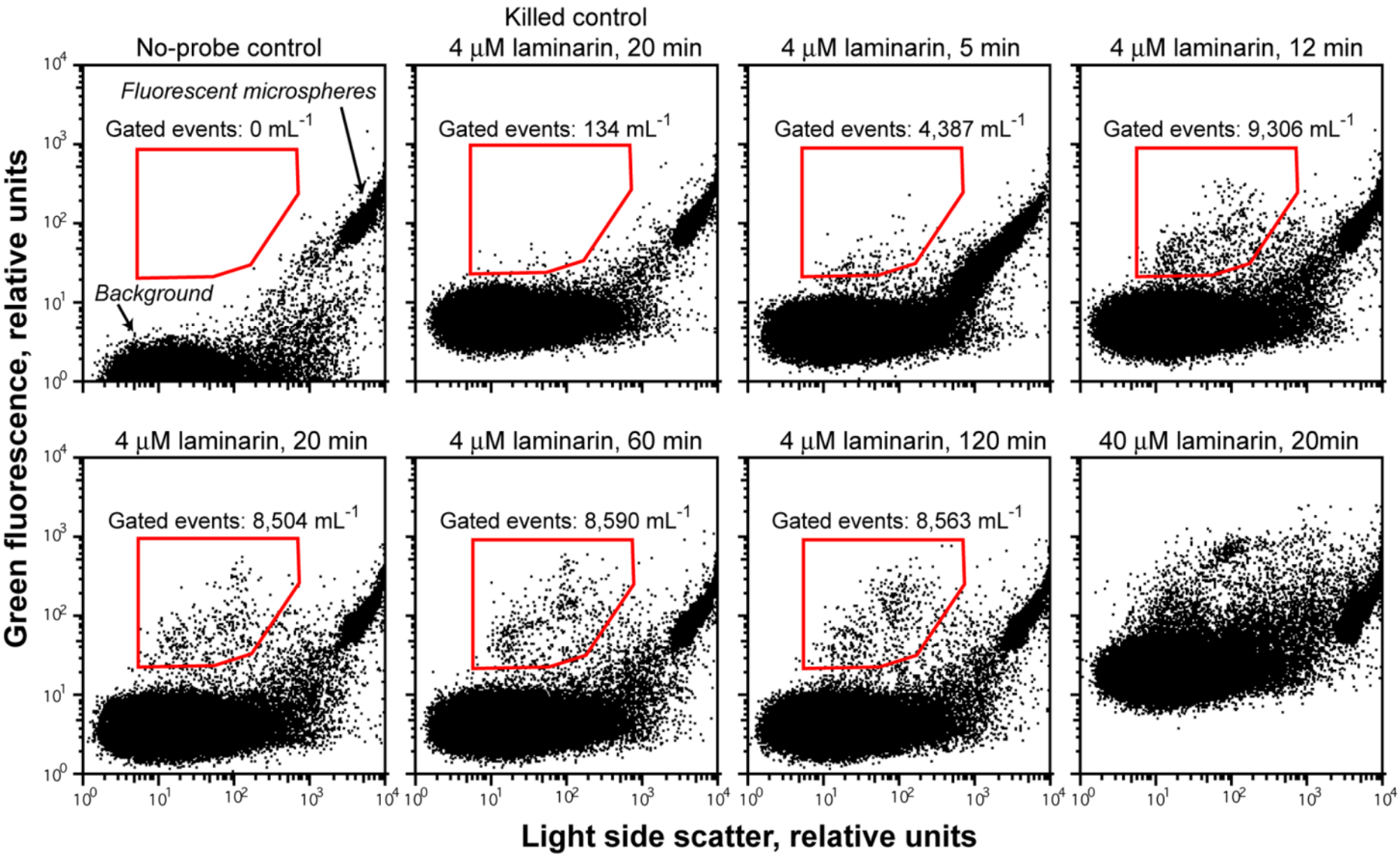
Fluorescence detected in single cells by flow cytometry after incubation

Putative polysaccharide-degrading bacteria



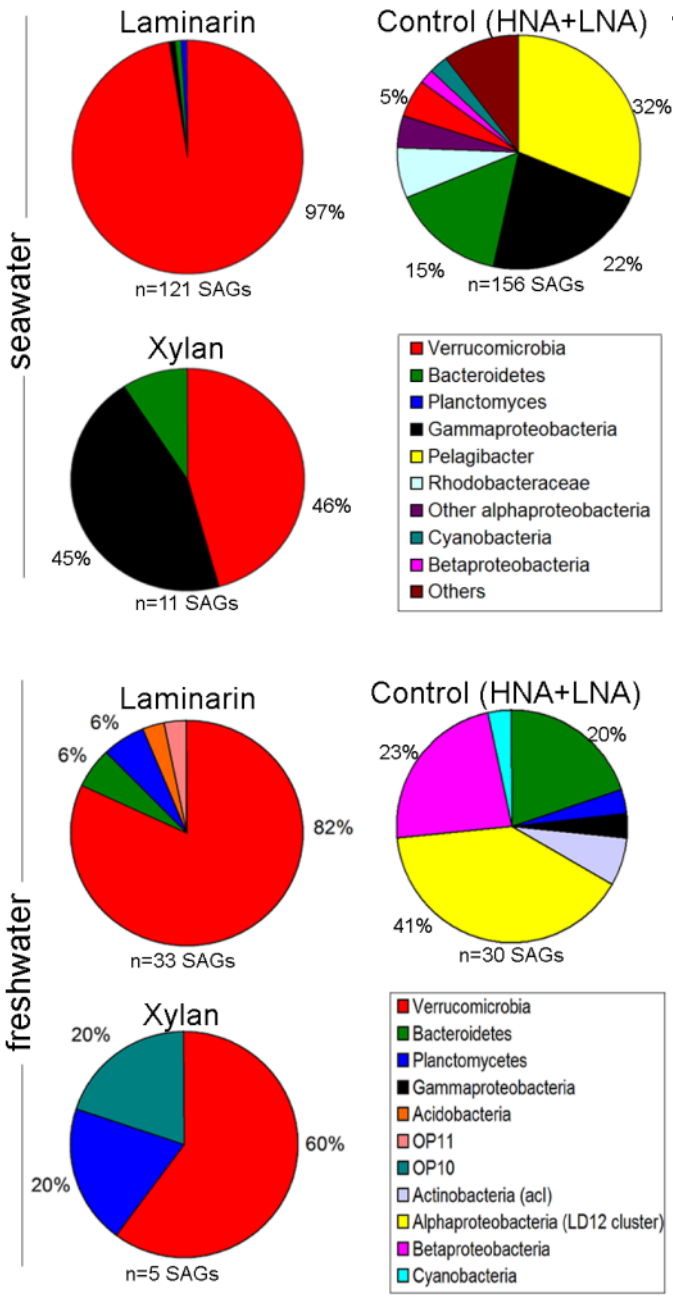


Optimization of the experiment

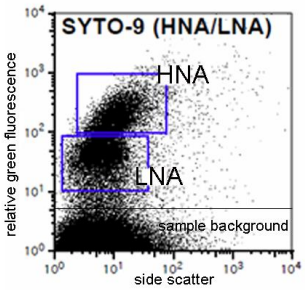


Samples incubated with fluorescently labeled laminarin and xylan

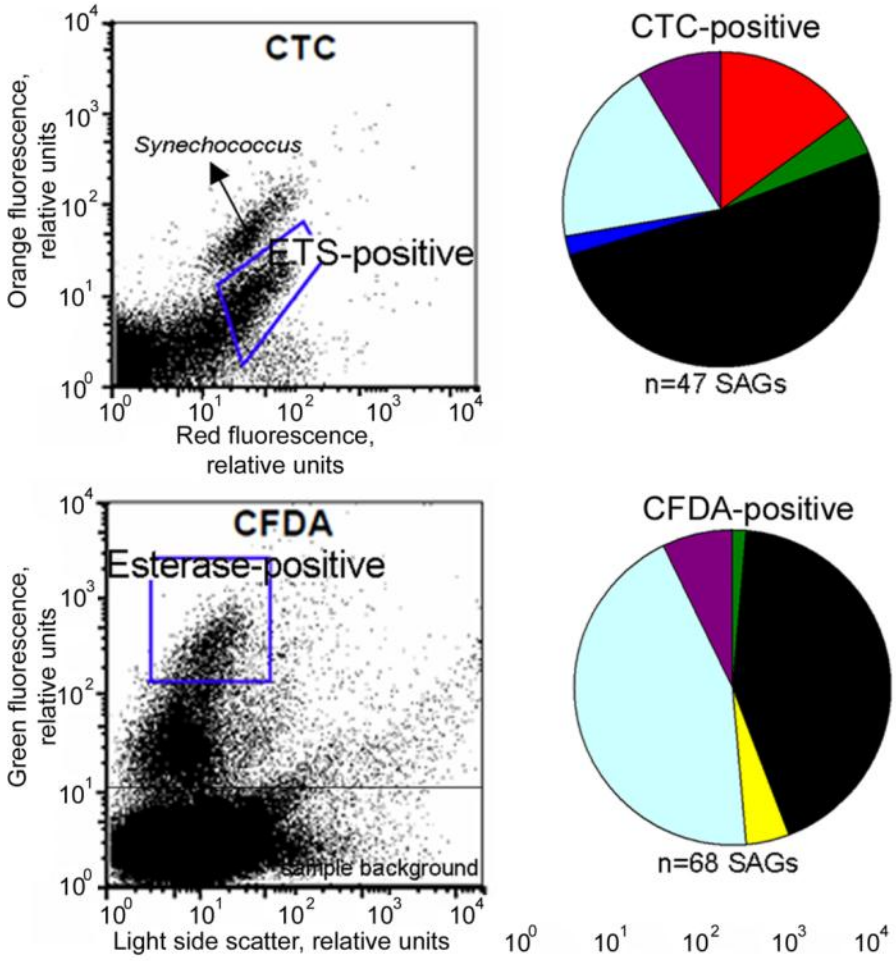
2000-5400 bacteria mL^{-1} involved in laminarin and xylan degradation



Control water not amended with polysaccharides



Key result:
Verrucomicrobia comprises 40-97% of total positive-labeled SAGs



Key result:
Verrucomicrobia is also active within the Bacterioplankton

Phylogenetic tree of positive-labelled Verrucomicrobia SAGs

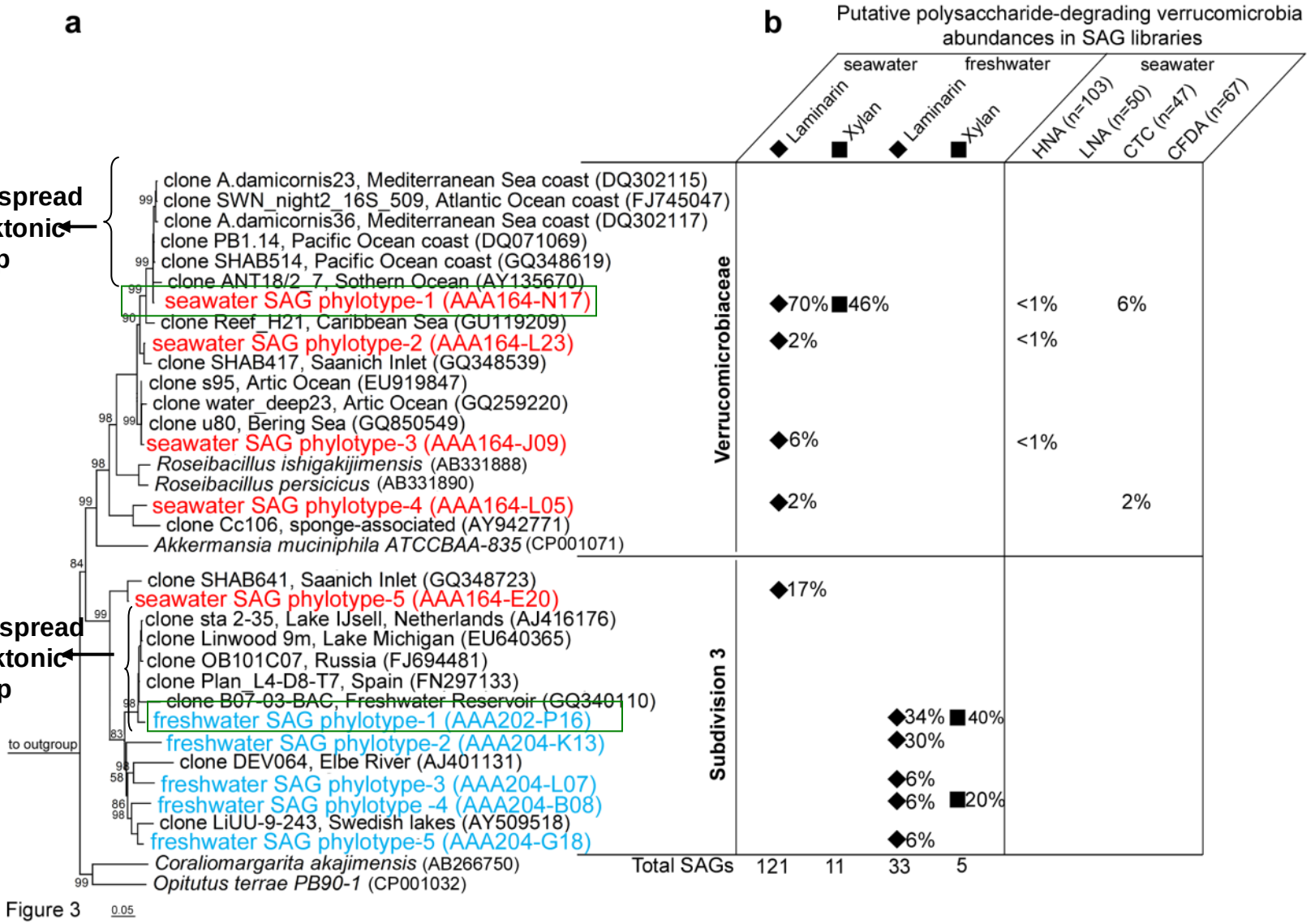


Figure 3

Phylogenetic tree of positive-labelled SAGs belonging to other groups

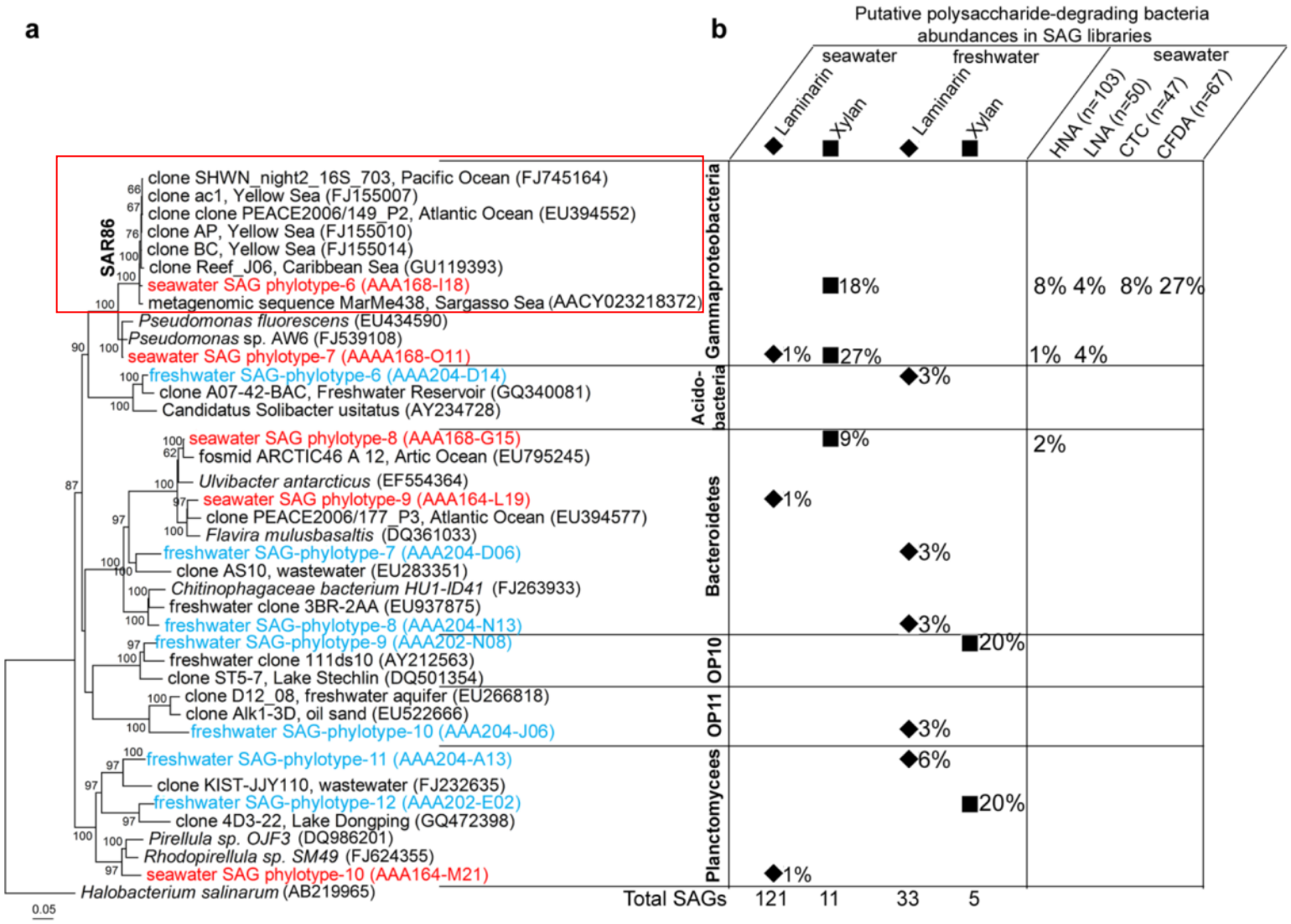
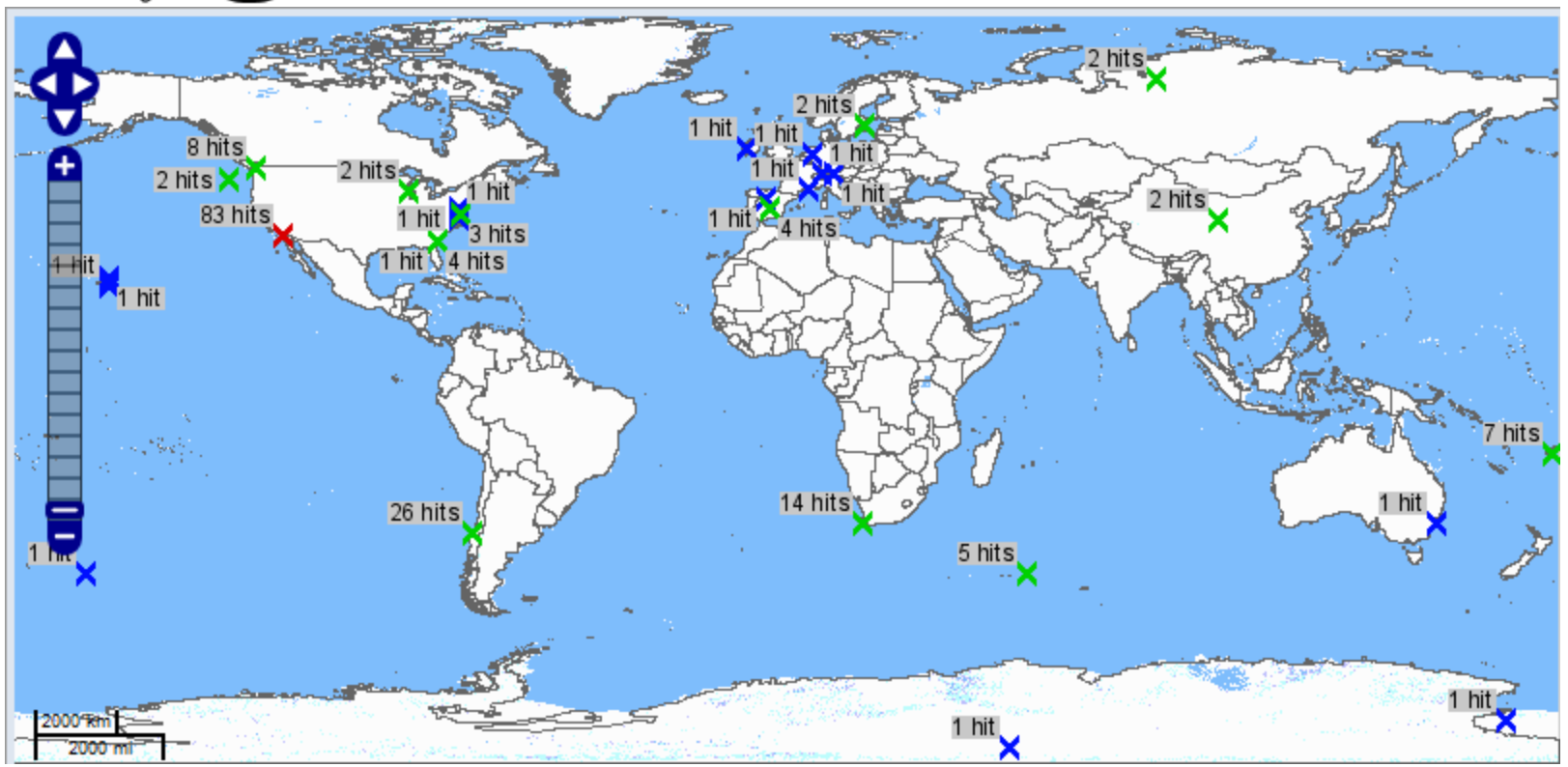


Figure 4

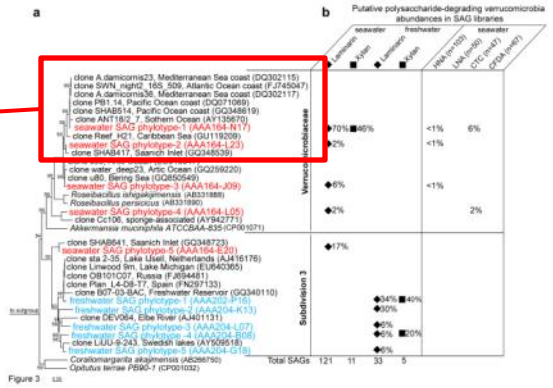
megx
marine ecological genomics

geographic-blast

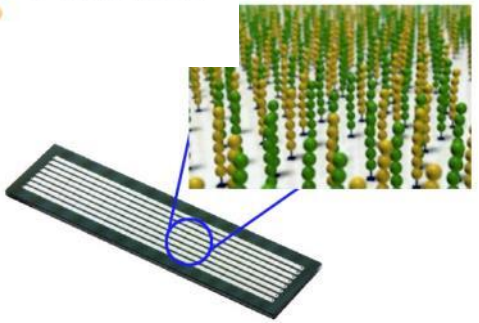
result



Sequencing an uncultured widespread coastal cluster



Sequencing SAGs by PacBio and Illumina…

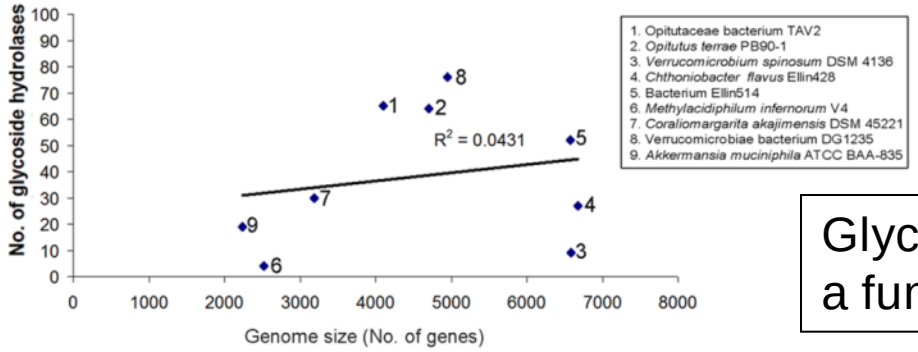


Supplementary Table 2. Assembly statistic

	AAA164-A21	AAA168-E21	AAA164-L15	AAA164-O14	AAA168-F10
Assembly size (bp)	1,011,436	2,060,559	2,641,408	3,346,464	4,850,044
Estimated genome recovery (%)					
Number of contigs	754	1084	710	1141	1397
Largest contig (bp)	34216	31946	116022	57159	92178
GC content (%)	48	49	49	49	49
Illumina sequencing effort (bp)					
Pac-Bio sequencing effort (Mbp)	328	476	429	170	430
Total predicted genes	920	1741	2237	2870	4157
No. of genes in KEGG/COG/Pfam					

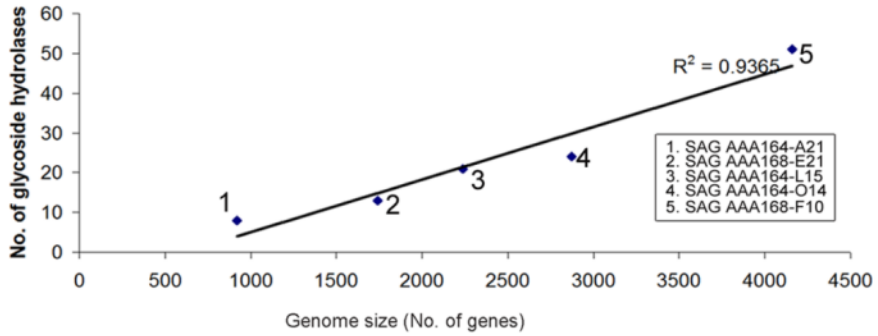
Estimated recovery: 88% for SAG AAA168-F10

Whole genome sequencing of the most predominant SAG phylotype

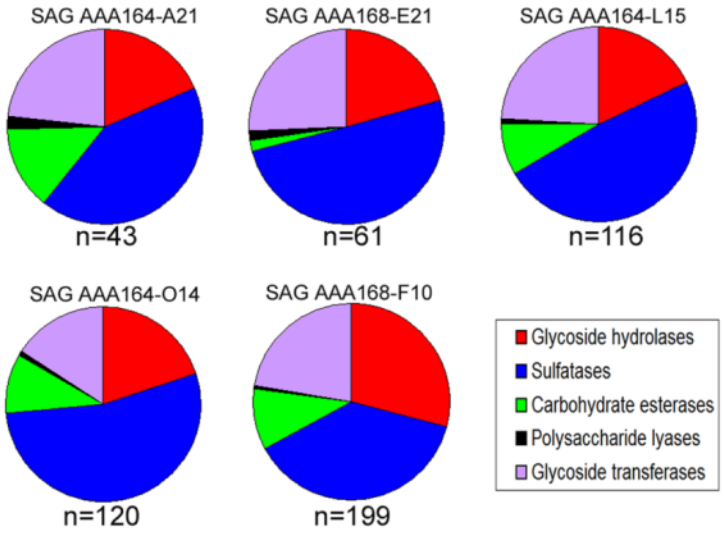


Glycoside hydrolysis is not a function of genome size

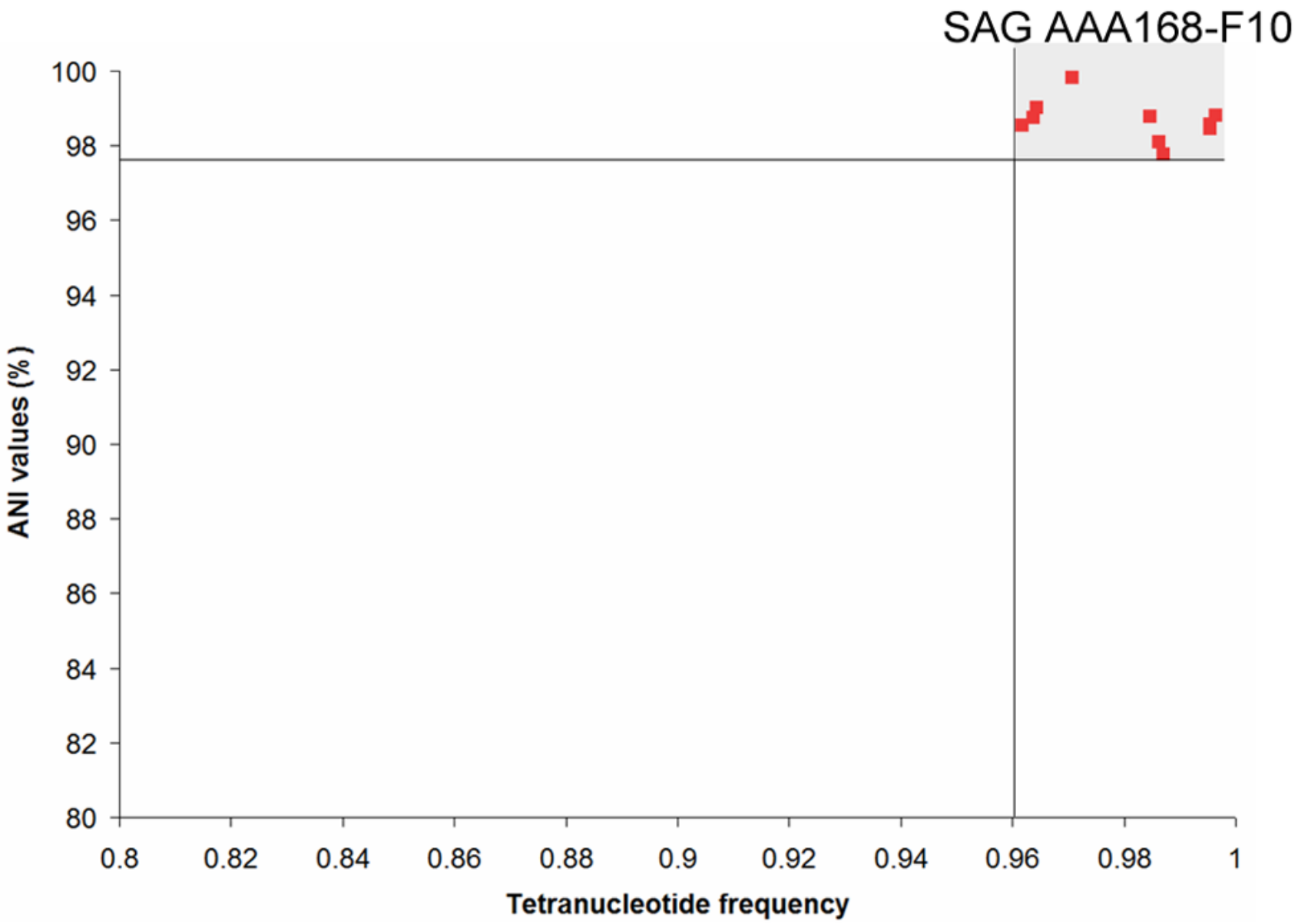
B



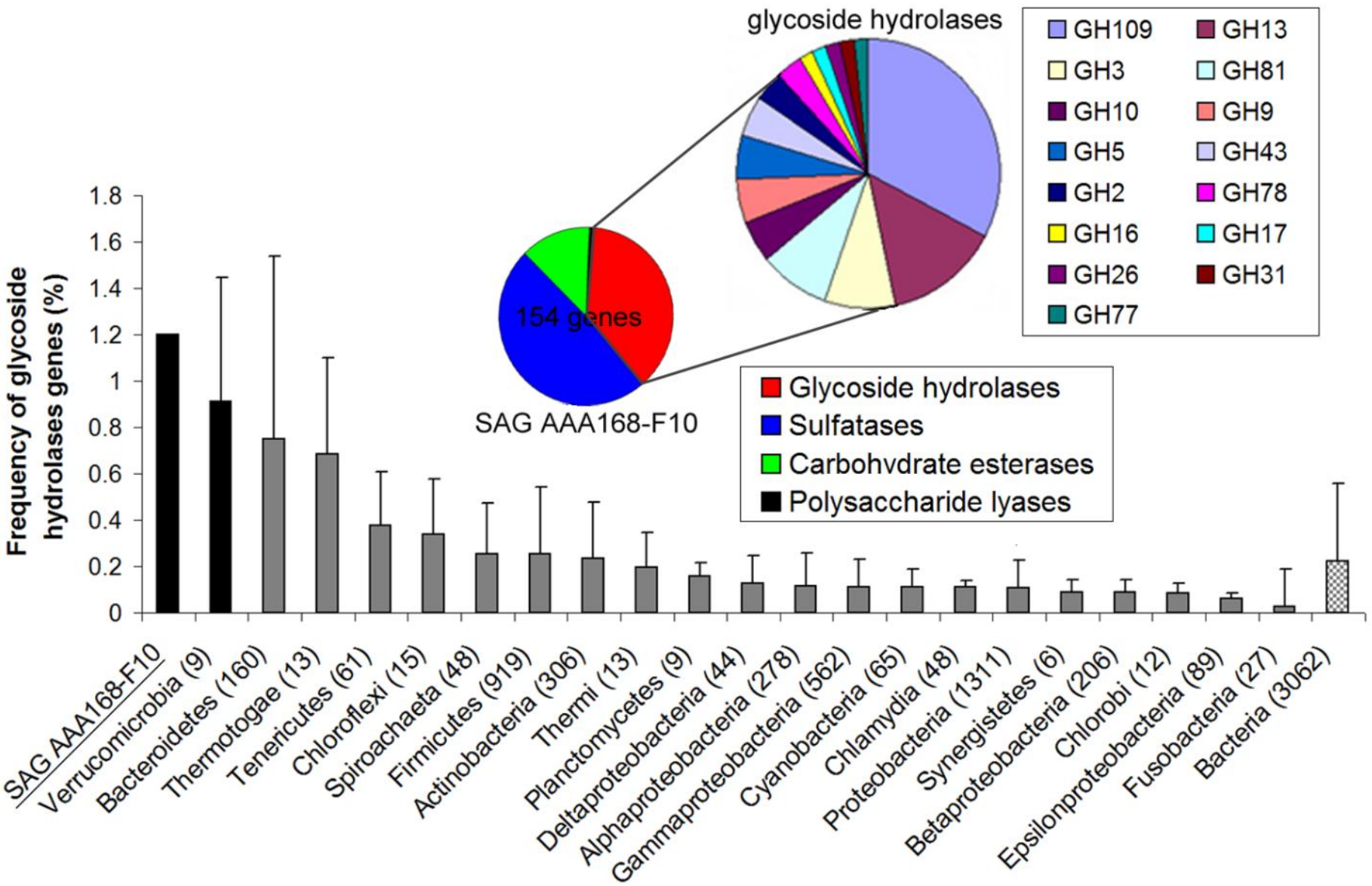
C



Genomic comparison among sequenced SAGs



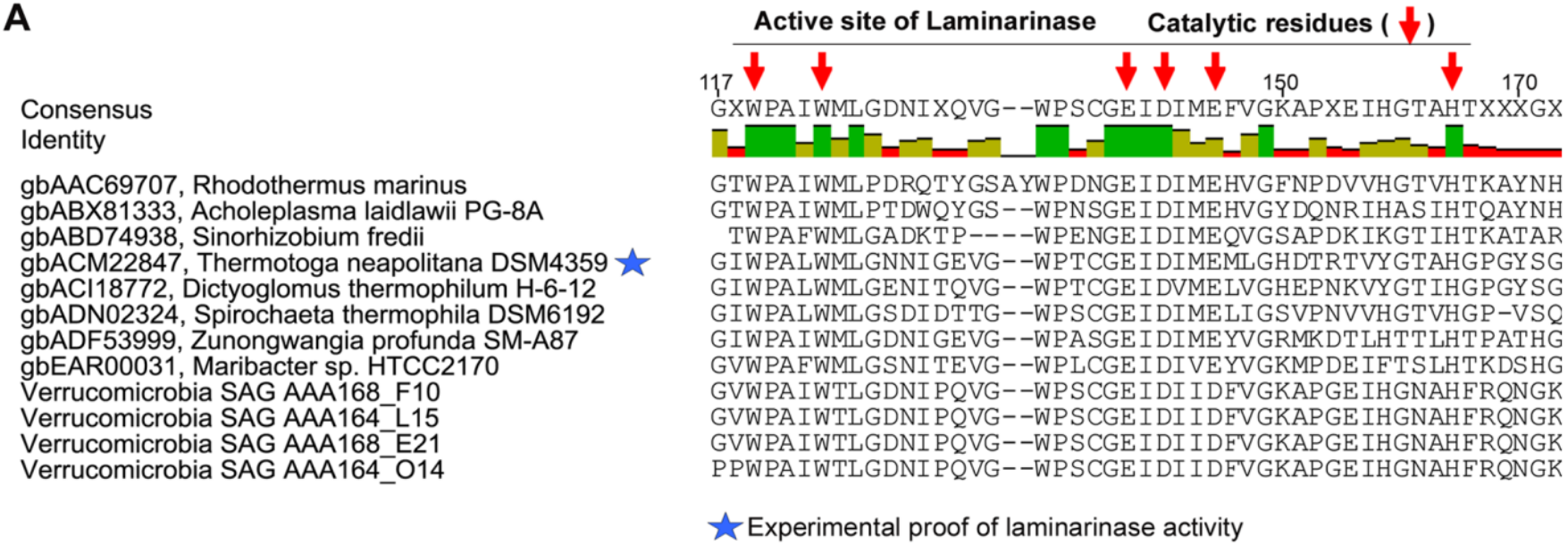
Role of Verrucomicrobia SAG in polysaccharide degradation



3062 analyzed genomes

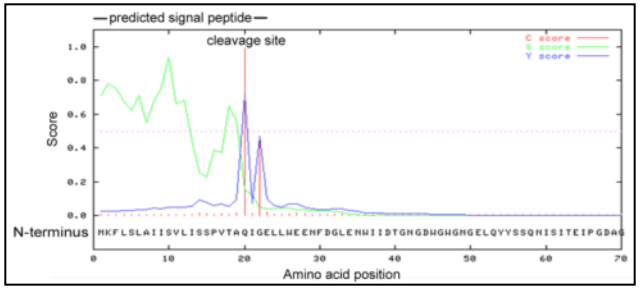
Going deeper in the potential polysaccharide degradation role…

A

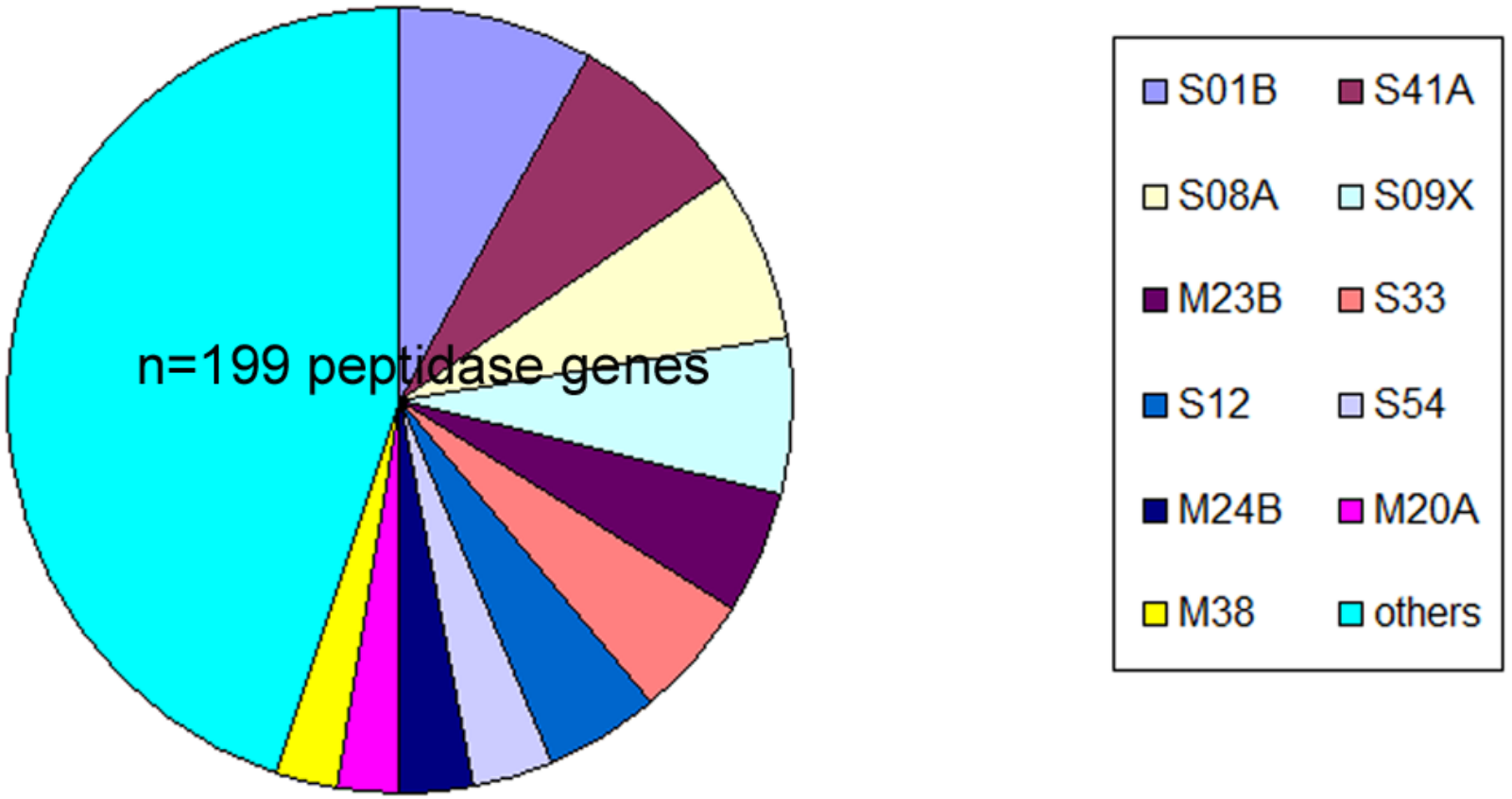


★ Experimental proof of laminarinase activity

B



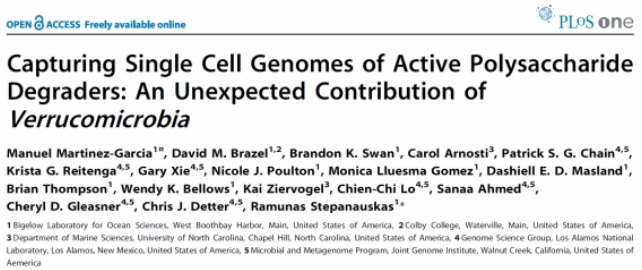
This verrucomicrobia is potentially involved in other biopolymer degradation



Conclusions:

1) Widespread planktonic *Verrucomicrobia* clusters with no prior knowledge on their ecological role contain putative laminarin and xylan degrading specialists that locally dominate this ecological niche –along with other bacterial groups- in marine and freshwater systems.

2) Single cell sequencing and comparative genomics suggest Verrucomicrobia as a major polysaccharide degrader in fresh and marine bacterioplankton



OPEN ACCESS Freely available online PLoS one

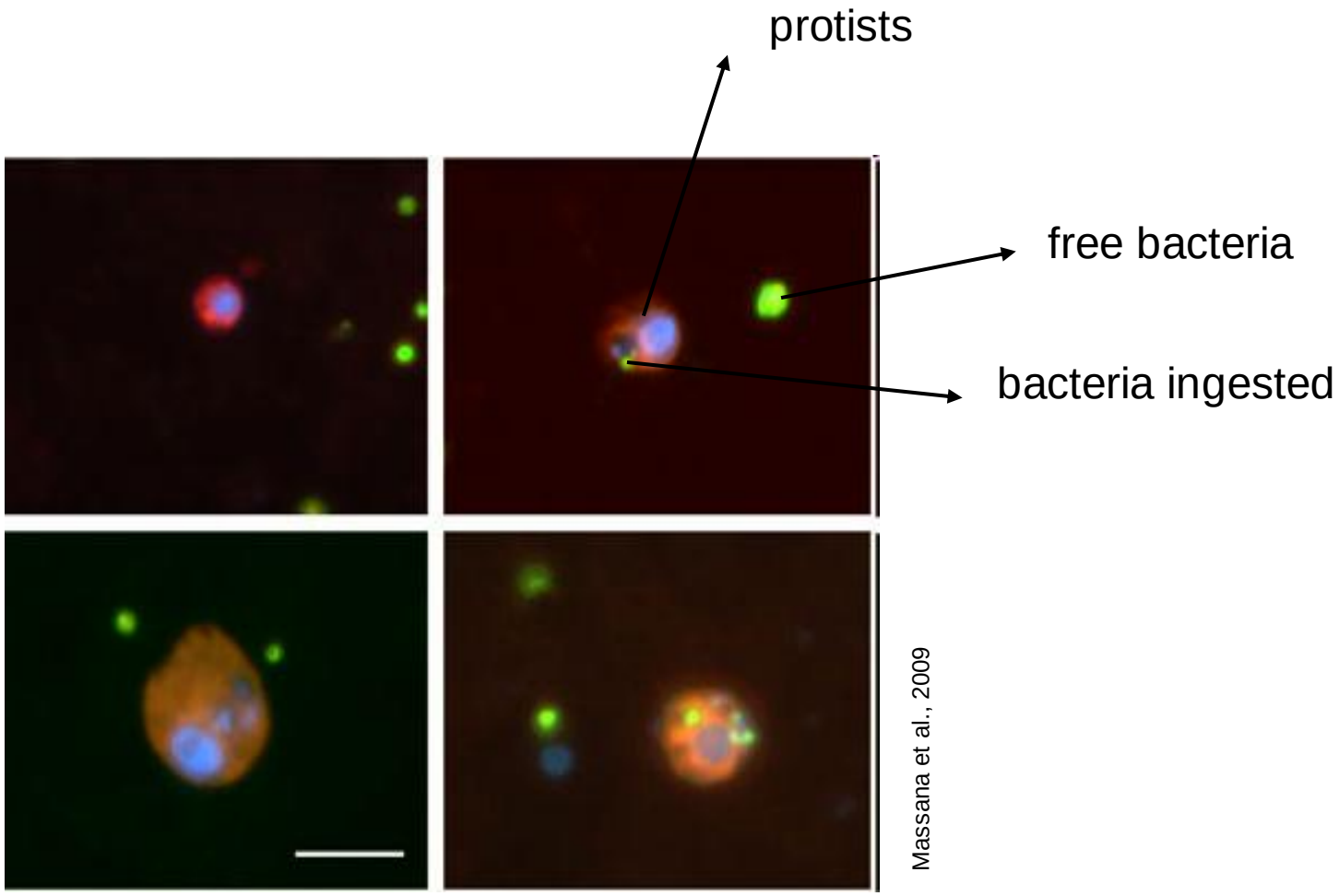
Capturing Single Cell Genomes of Active Polysaccharide Degraders: An Unexpected Contribution of *Verrucomicrobia*

Manuel Martinez-Garcia[1¤], David M. Brazel[1,2], Brandon K. Swan[1], Carol Arnosti[3], Patrick S. G. Chain[4,5], Krista G. Reitenga[4,5], Gary Xie[4,5], Nicole J. Poulton[1], Monica Lluesma Gomez[1], Dashiell E. D. Masland[1], Brian Thompson[1], Wendy K. Bellows[1], Kai Ziervogel[3], Chien-Chi Lo[4,5], Sanaa Ahmed[4,5], Cheryl D. Gleasner[4,5], Chris J. Detter[4,5], Ramunas Stepanauskas[1]*

1 Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Main, United States of America, 2 Colby College, Waterville, Main, United States of America, 3 Department of Marine Sciences, University of North Carolina, Chapel Hill, North Carolina, United States of America, 4 Genome Science Group, Los Alamos National Laboratory, Los Alamos, New Mexico, United States of America, 5 Microbial and Metagenome Program, Joint Genome Institute, Walnut Creek, California, United States of Aemerica

PART 2

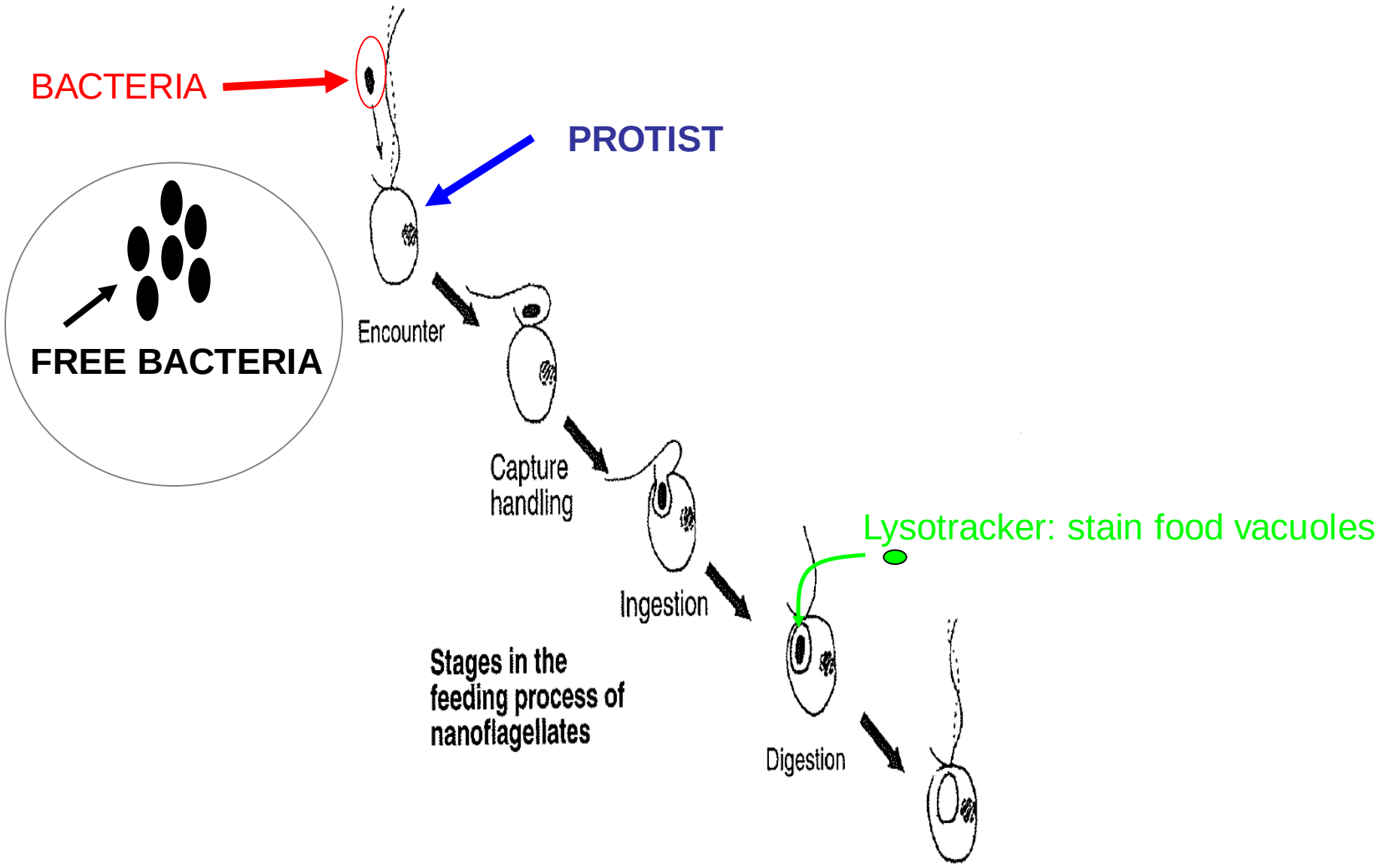
Unveil the ecological interaction between protists and bacteria in natural assemblages: predation and symbiosis



"Very cool" image showing the interaction between MAST-4 and bacteria

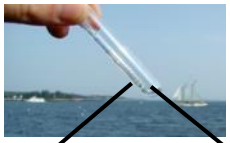
However, identification of both the protistan grazer and the bacterial prey has been shown only in the lab and never in natural assemblages

☆ *Main goal of the project*



Modified from Jurgens and Matz, 2002

Dock sample



1. Sort protists actively grazing

2. Identify protists by 18S rRNA gene

3. Identify the bacterial prey by 16S rRNA gene

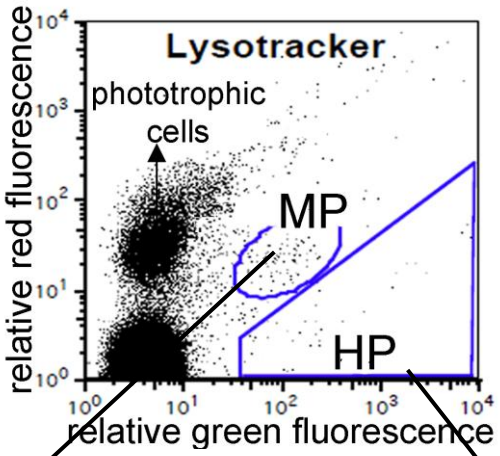
1. Sort free bacterioplankton

2. Identify the free bacterioplankton

comparison

Targeting protists actively grazing on bacterioplankton

Lysotracker (food vacuoles) green fluorescence

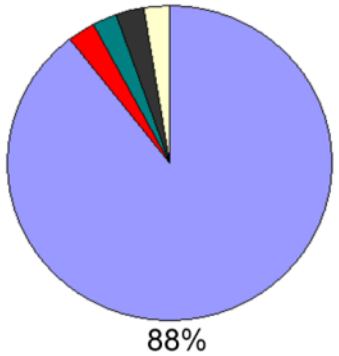


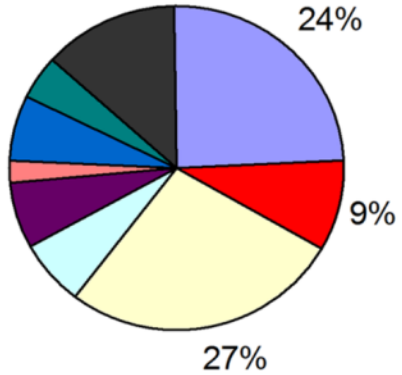
Two protist populations

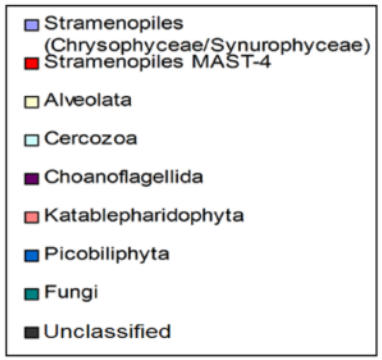
Mixotrophic protists (MP) n=38 SAGs

Heterotrophic protists (HP) n=47 SAGs

Eukarya

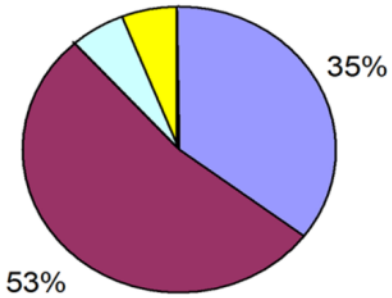




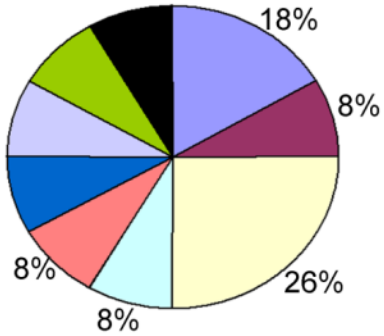


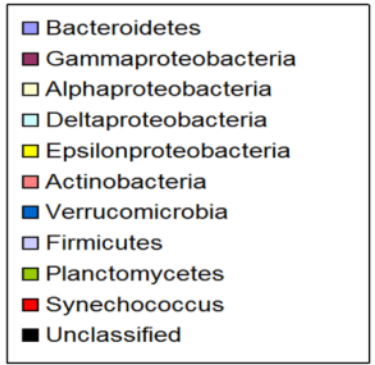
***Bacteria* in single protist cells**

n=17 SAGs

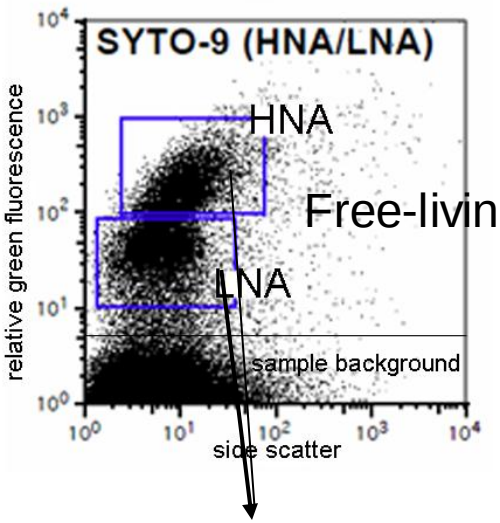


n=12 SAGs



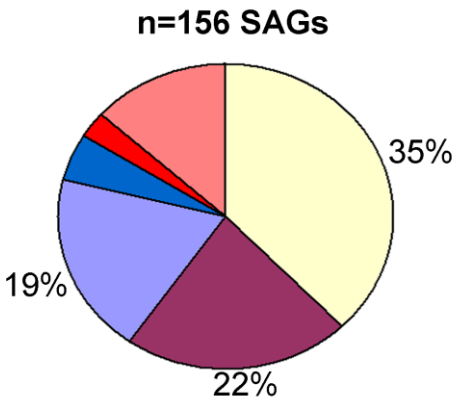


Targeting free-living bacterioplankton

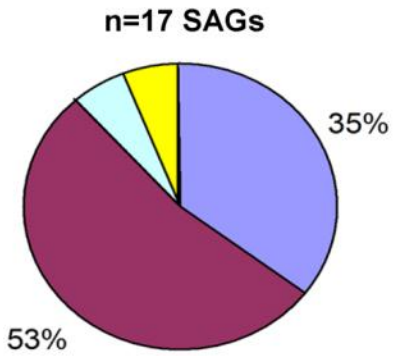


Free-living bacterioplankton

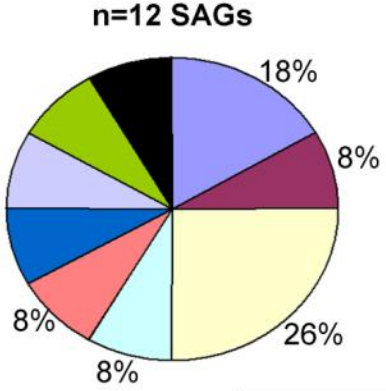
Bacterioplankton

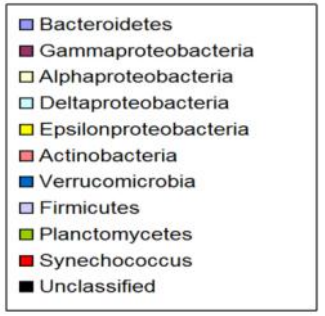


Bacterial prey
in mixotrophic protists



Bacterial prey
in heterotrophic protists



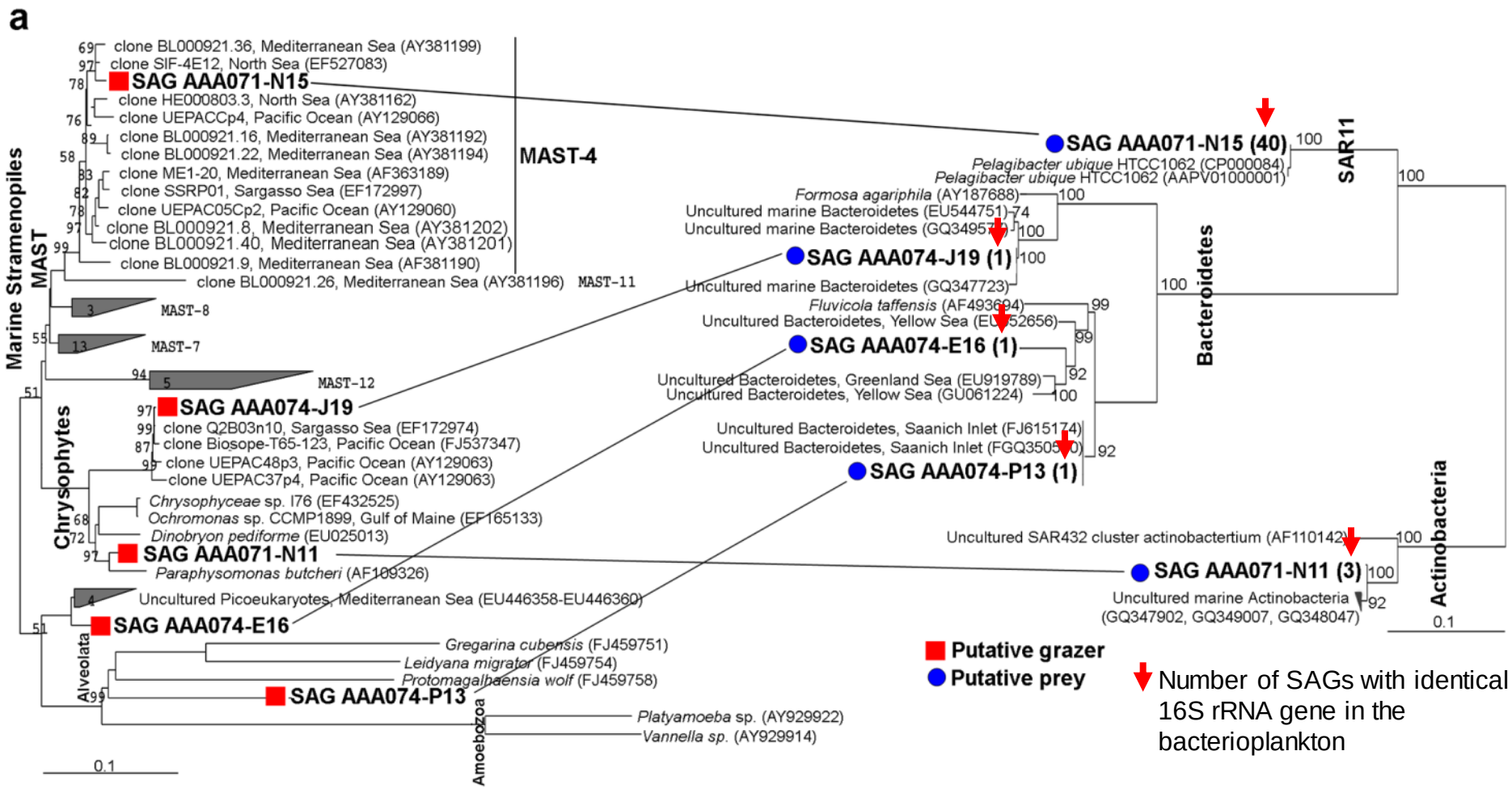


Comparing free-living bacterioplankton and putative bacterial preys

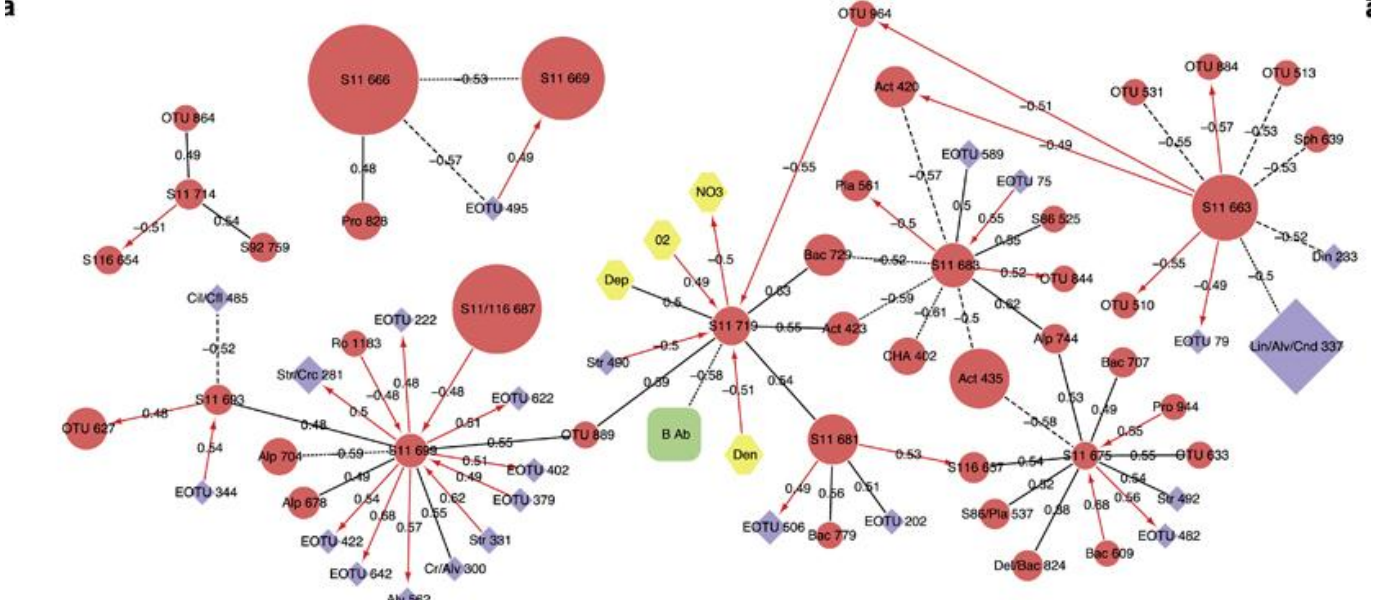
α selectivity index for
predator food preference (Gammaproteobacteria)=0.8

Protists (18S rRNA gene)

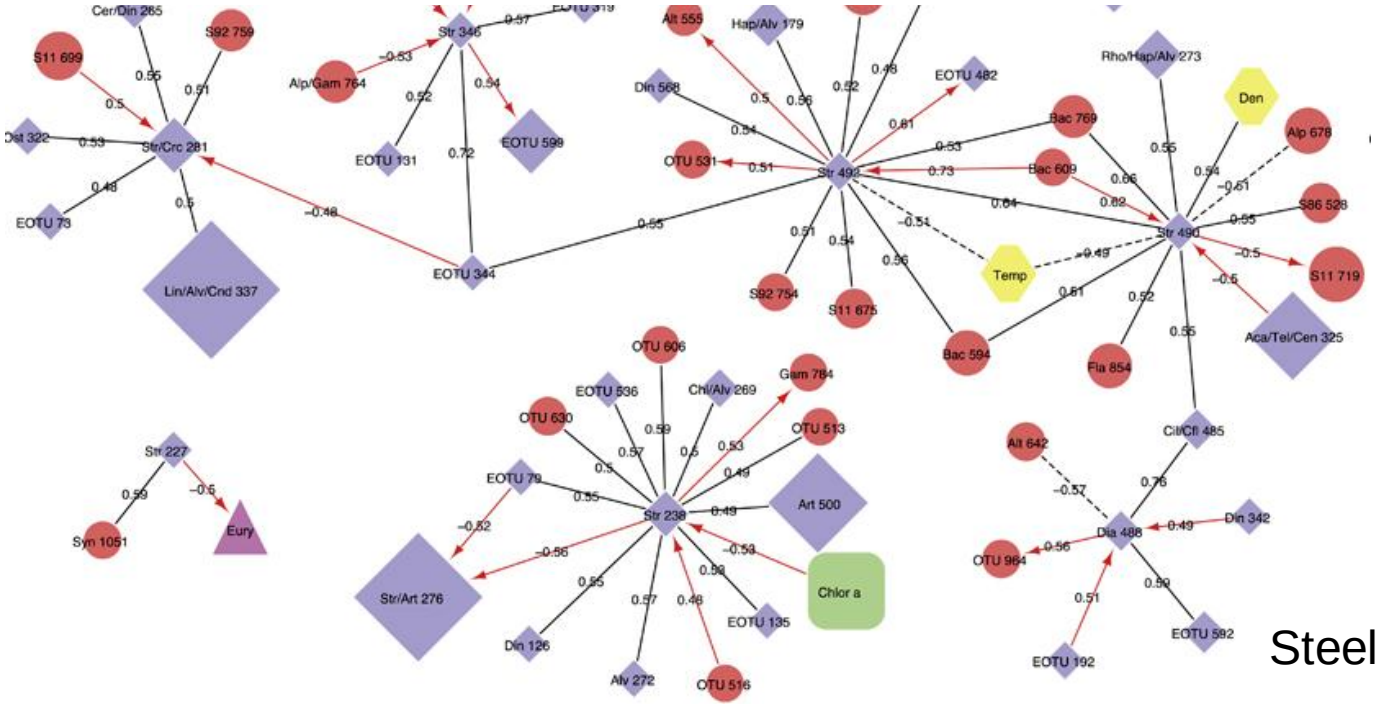
Bacteria (16S rRNA gene)



Linking both the identity of the prey and the predator



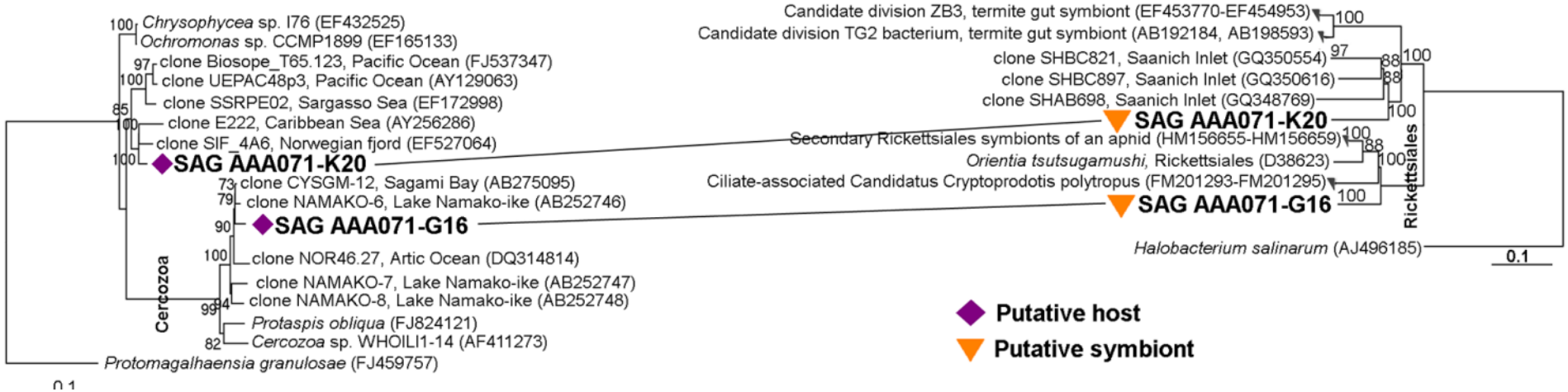
SAR 11 *(Pelagibacter ubique)* is highly connected with MAST groups in coastal areas
Negative correlation indicates likely predation



Steele et al. 2011

Protists (18S rRNA gene)

Bacteria (16S rRNA gene)



New way to identify putative symbionts and hosts

PART 2
Unveiled the ecological interaction between protists and bacteria in natural assemblages: predation and symbiosis

Conclusions:

1) The most abundant marine protist group (MAST-4) putatively grazing on the ubiquitous *Pelagibacter* ubique!!

2) Revealed the identity of protists grazing on widespread marine *Bacteroidetes* and *Actinobacteria*

3) *Gammaproteobacteria* as preferred prey in mixotrophic protists

4) Discovered new putative symbionts in Cercozoa and Crysophyta groups

The ISME Journal (2011), 1–5
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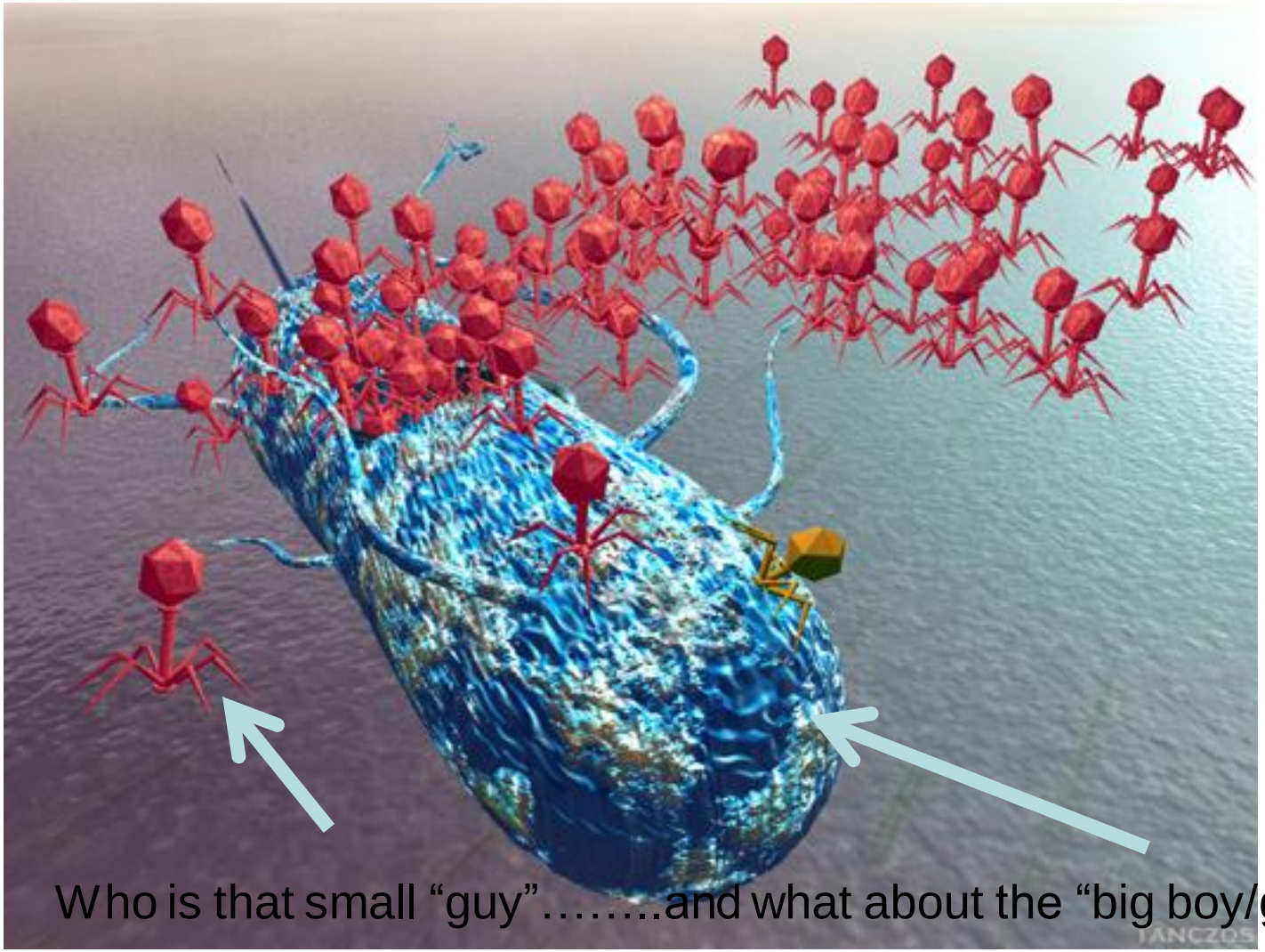
SHORT COMMUNICATION

Unveiling *in situ* interactions between marine protists and bacteria through single cell sequencing

Manuel Martinez-Garcia[1], David Brazel[1,2], Nicole J Poulton[1], Brandon K Swan[1], Monica Lluesma Gomez[1], Dashiell Masland[1], Michael E Sieracki[1] and Ramunas Stepanauskas[1]
[1]*Single Cell Genomics Center, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA and* [2]*Colby College, Waterville, ME, USA*

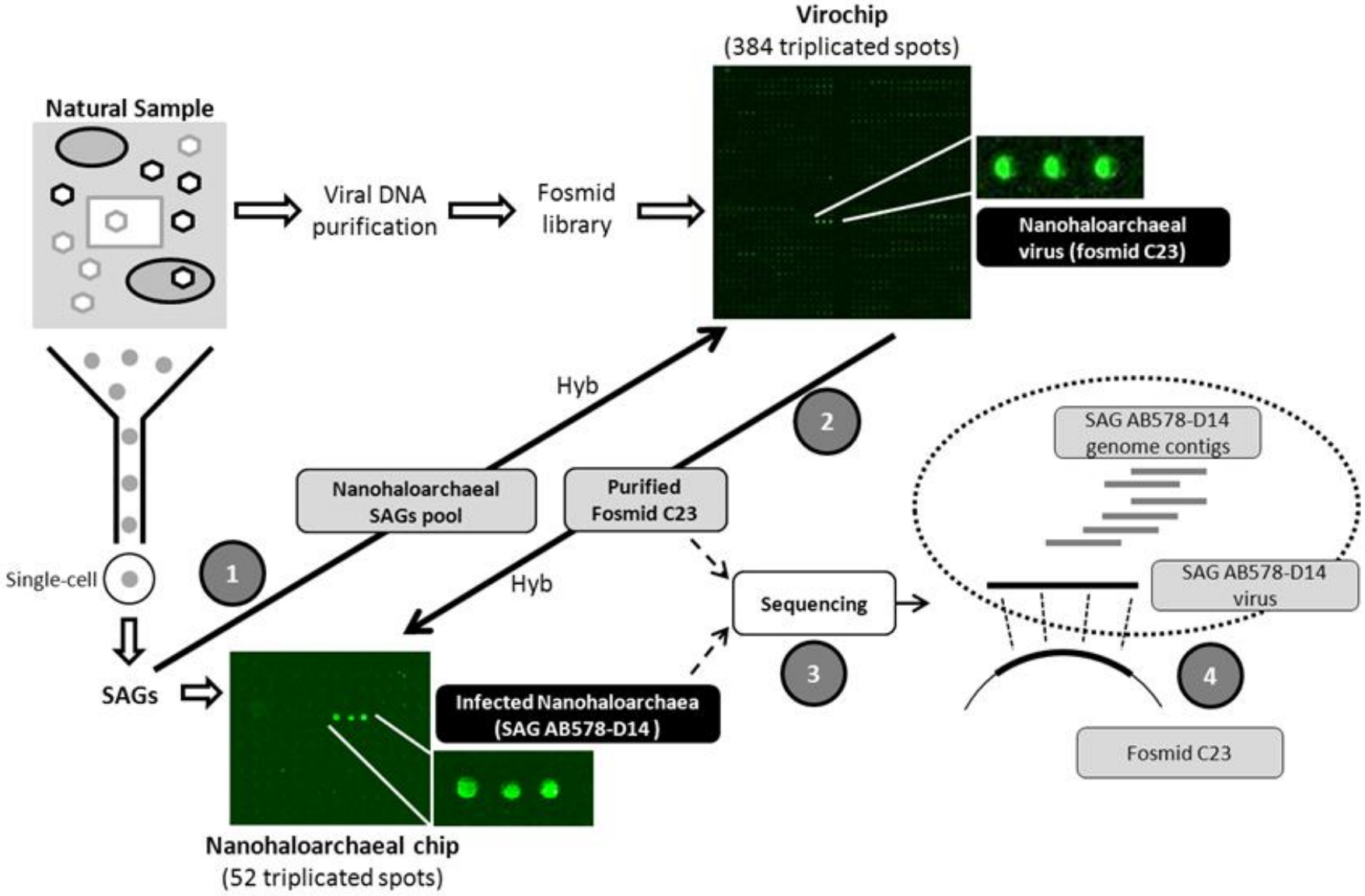
Part 3
Assign virus to host in natural uncultured assemblages: one at a time

Manuel Martínez-García*, Fernando Santos*, Mercedes Moreno-Paz, Víctor Parro, and Josefa Antón
*equally contribution

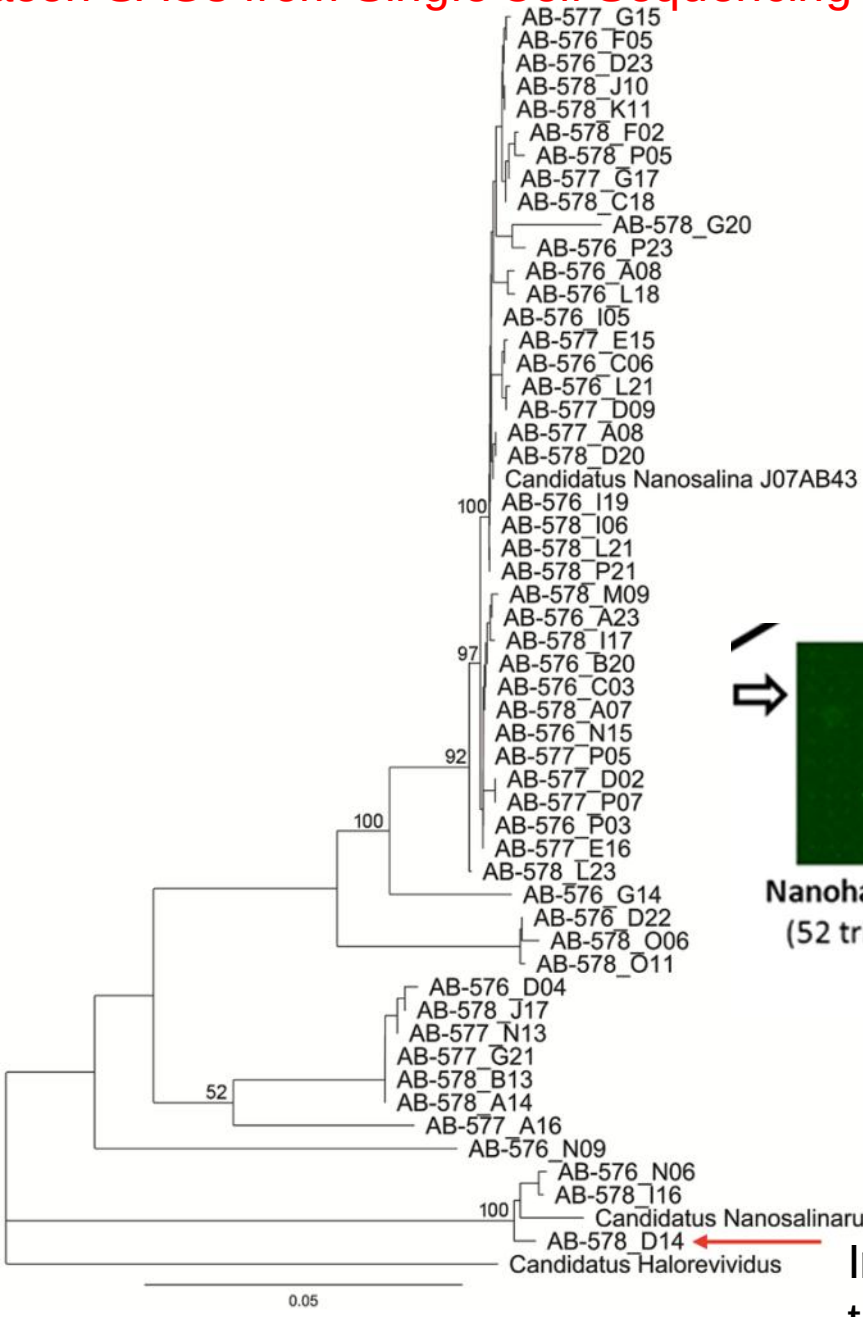


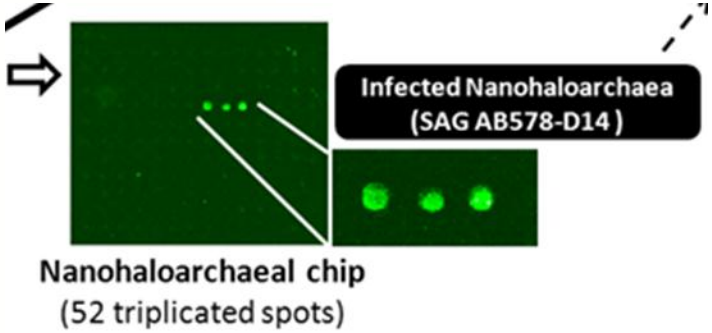
Easy when you can culture the pair….

Combining Single Cell genomics and Microarray technology: a new approach to Assign virus to host in uncultured natural assemblages



52 nanohaloarchaeon SAGs from Single Cell Sequencing (CR30)





Infected SAGs: positive in the microarray

Sequencing both the host and the virus

Table S1. Illumina MiSeq sequencing results for the SAG AB578-D14 and the corresponding viral fosmid C23

	No. of total reads	Quality score (% of total reads) Q30	Q20	Mean read length (bp)
Host AB578-D14	8,330,570	92.6	89.8	179
Virus (fosmid insert)	11,911,500	92.5	89.5	190

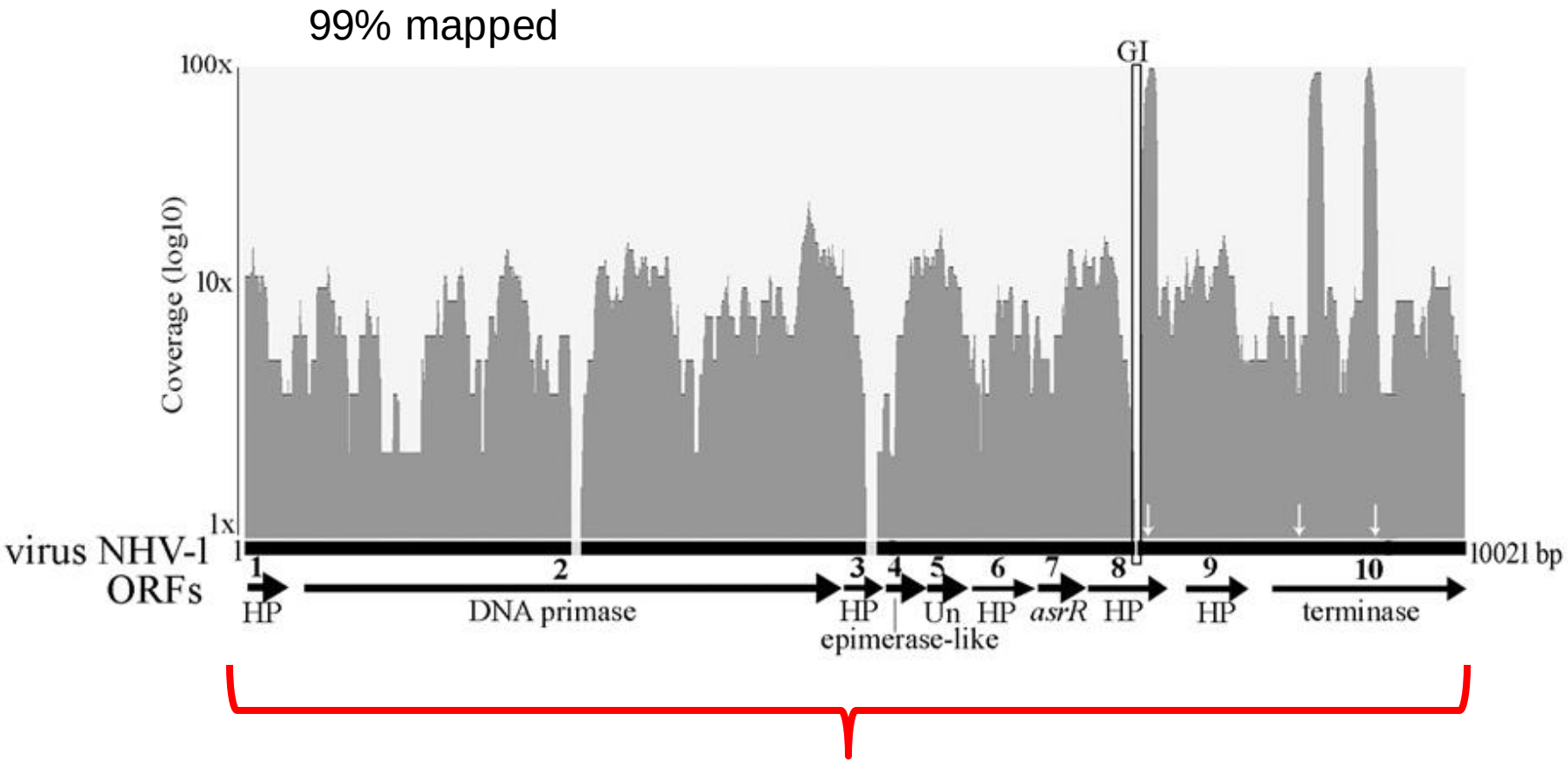
Table S3. Assembly comparison of performance of SPAdes, CAMERA meta-assembler+Geneious R6.1 and VELVET assemblers (*36-38*) for the nanohaloarchaeon host D14

Assembler	No. total contigs	No. contigs >1000 bp	Total nucleotide assembled	Max. length contig (bp)	NG50* value	N50±
SPAdes	962	112	1028544	54146	2741	9219
CAMERA Meta-assembler + Geneious R6.1 assembler	263	106	477107	39523	<200	1722
VELVET	332	62	209361	17300	<200	2766

*Since assembly sizes from the three strategies was very uneven (0.2-1 Mbp), the NG50 statistics was used to compare the three resulting assemblies. The NG50 statistic is the same as the N50 except that the genome size was used rather than the assembly size. Genome size used here for normalization was that from SPAdes.

±Only contigs of 500 bp and longer were taken in consideration for N50 estimation

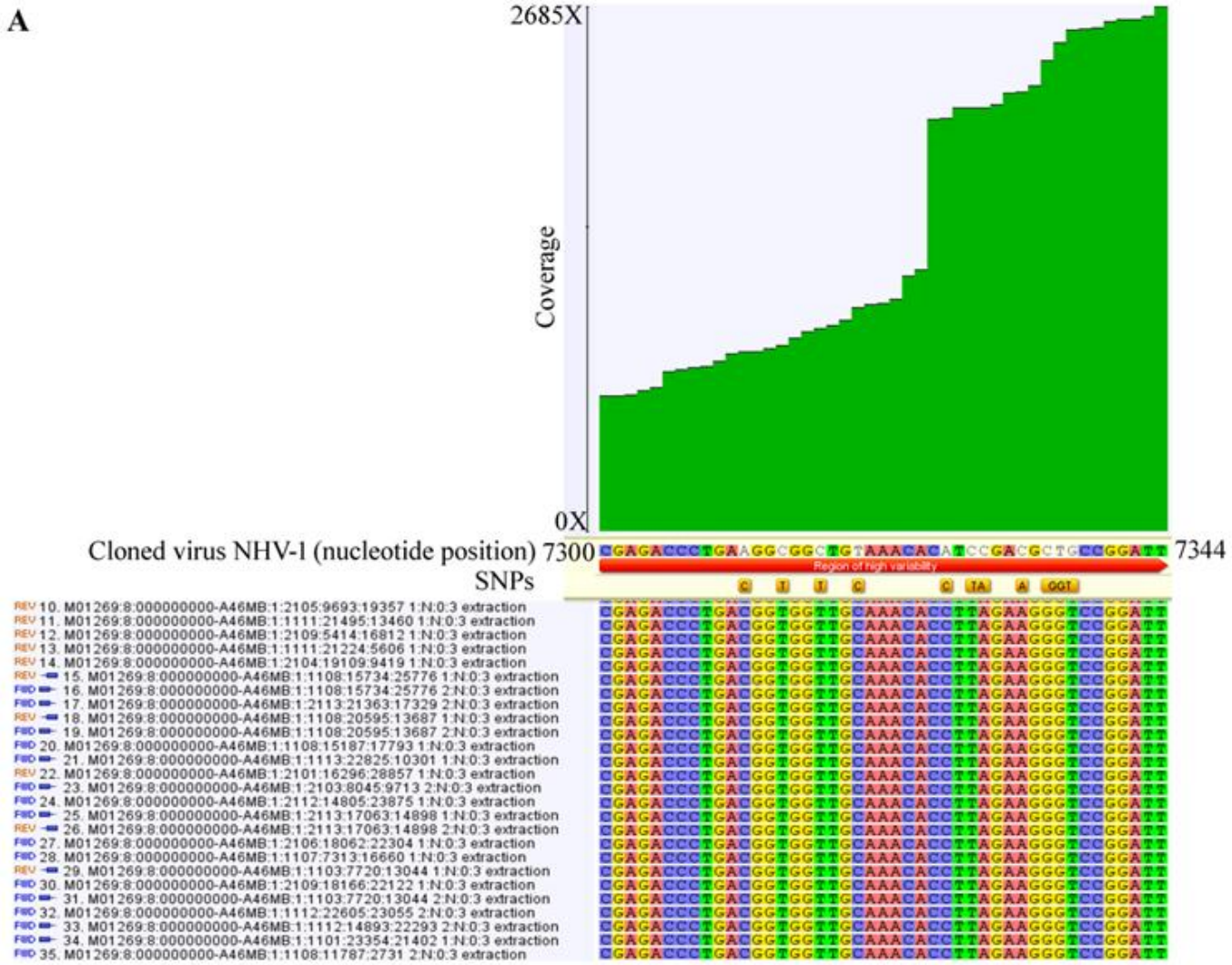
Mapping reads from the single nanohaloarchaeon cell onto the cloned virus



Assembly of virus cloned in fosmid

Most viral ORFs were related to ORFs from García-Heredia et. (2011) al and Santos et al. (2010,2011)

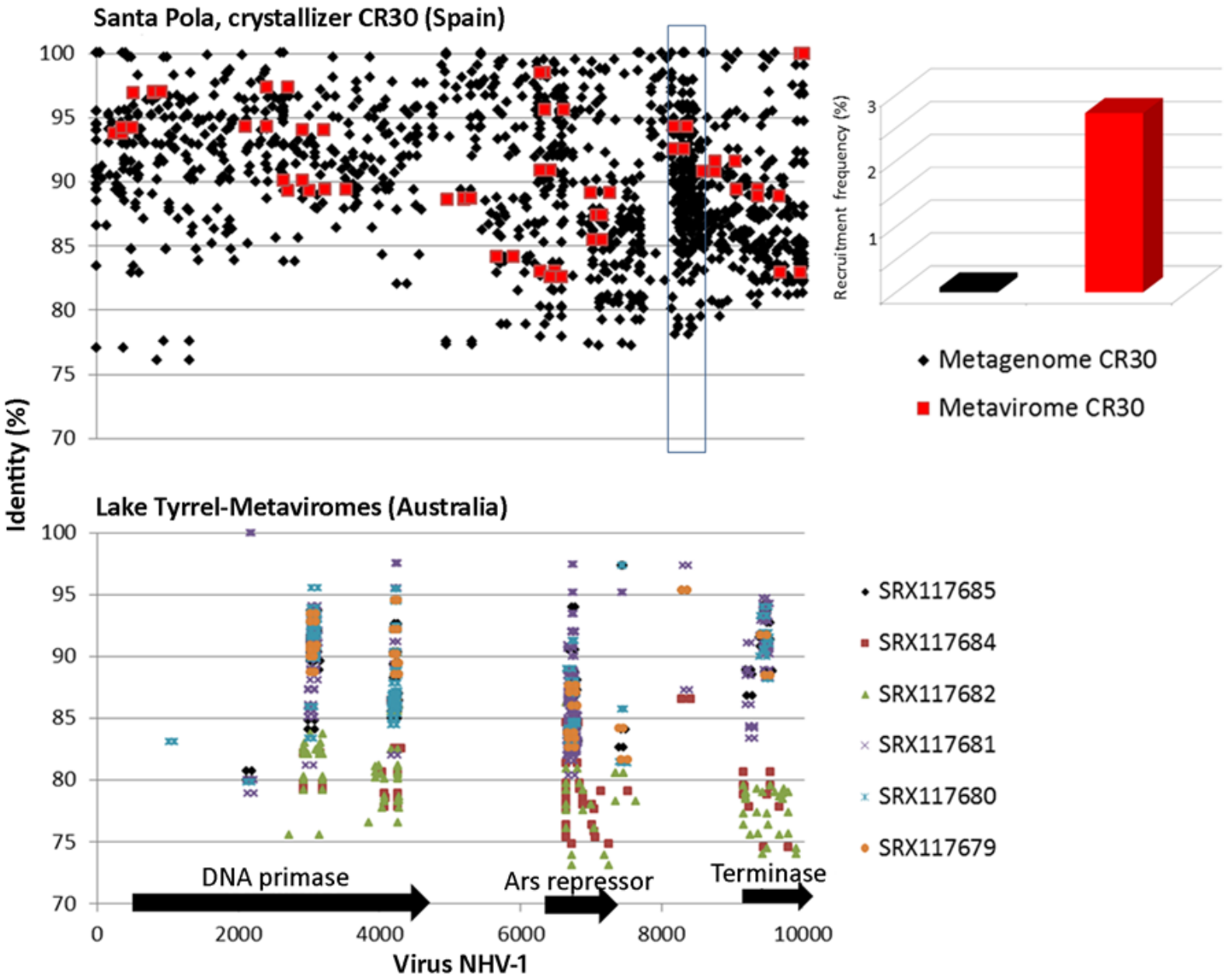
Virus NHV-1: two quasispecies



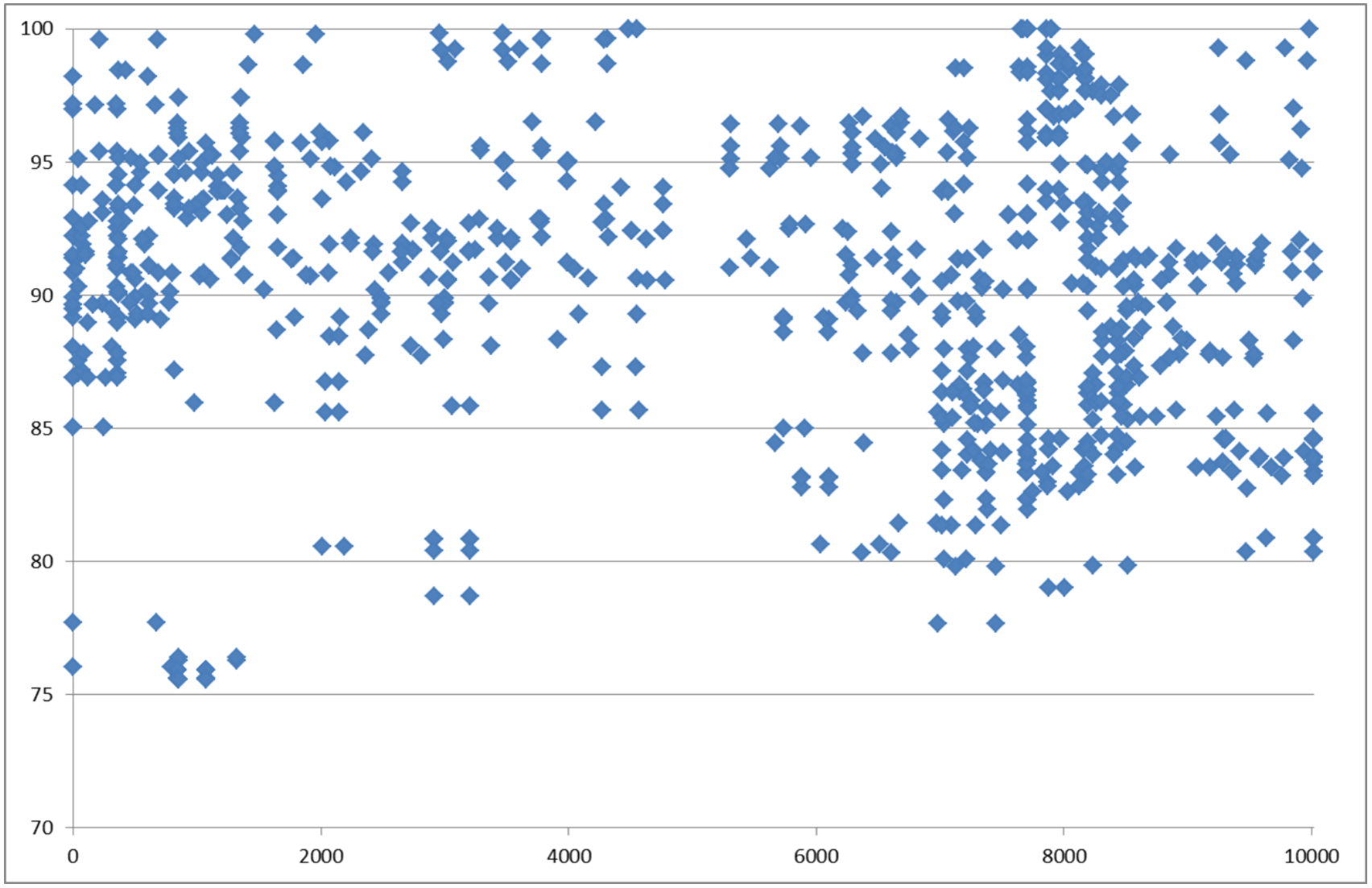
Virus NHV-1 quasispecie A (cloned virus) CGAGACCCTGA*A*GG*C*GG*C*TG*T*TAAACAC*A*T*CC*GA*C*G*CTG*

Virus NHV-1 quasispecie B (from SAG DI4) CGAGACCCTGA*C*GG*T*GG*T*TG*C*TAAACAC*C*T*TA*GA*A*G*GGT*

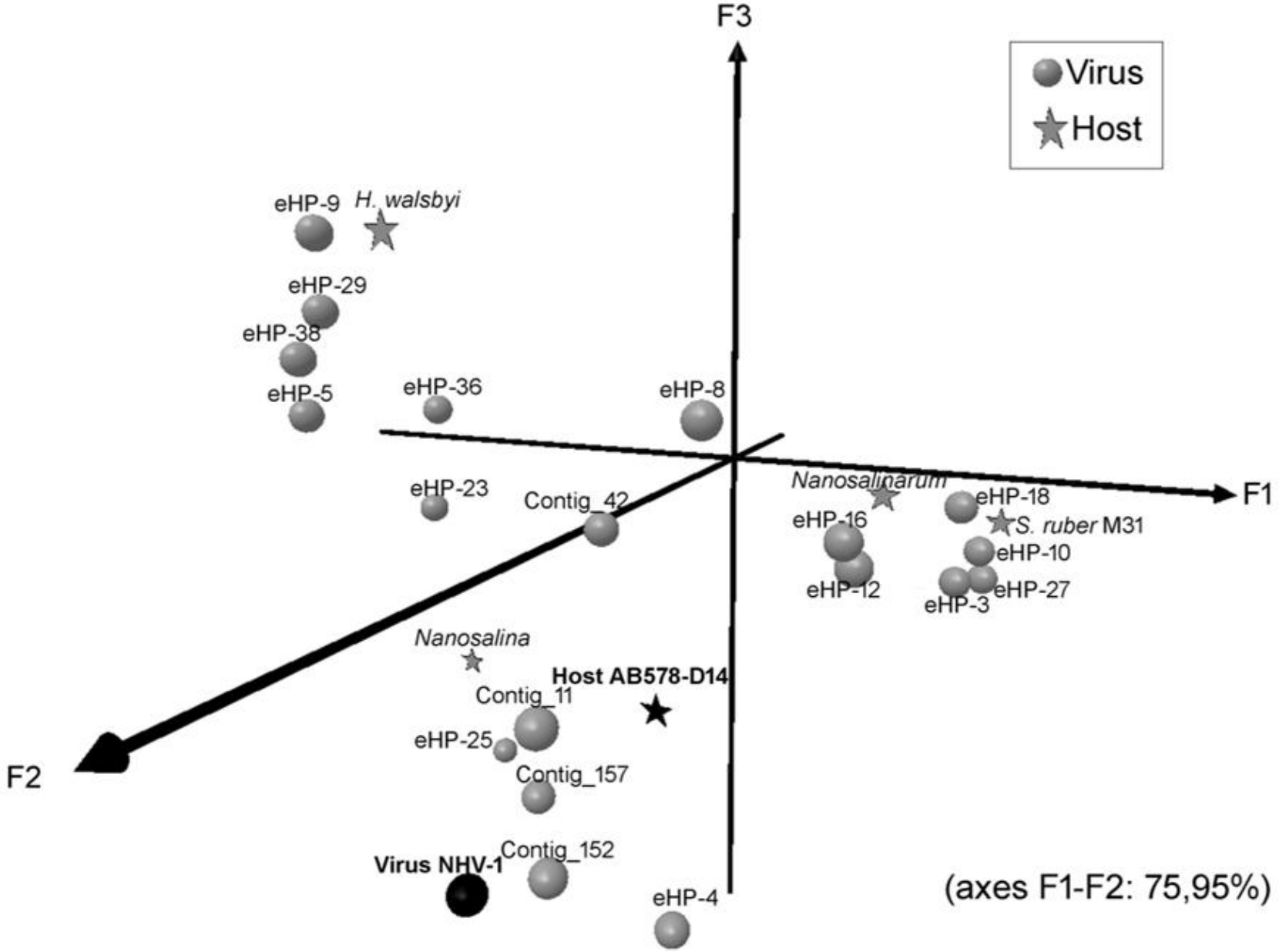
Virus NHV-1 is a *“common”* virus (*is not that weird…*)



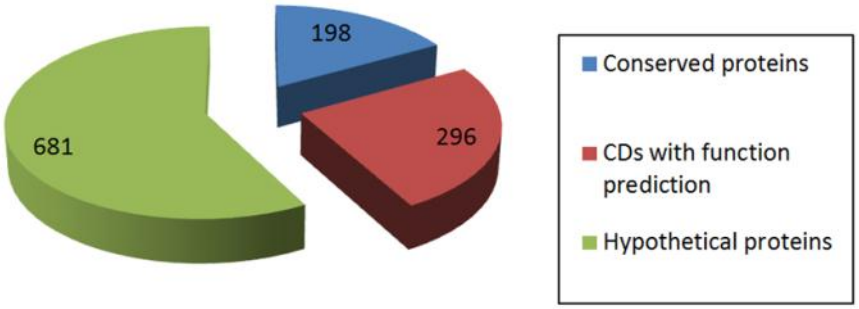
Recruitment of virus NHV-1 against a 454 metavirome from CR30



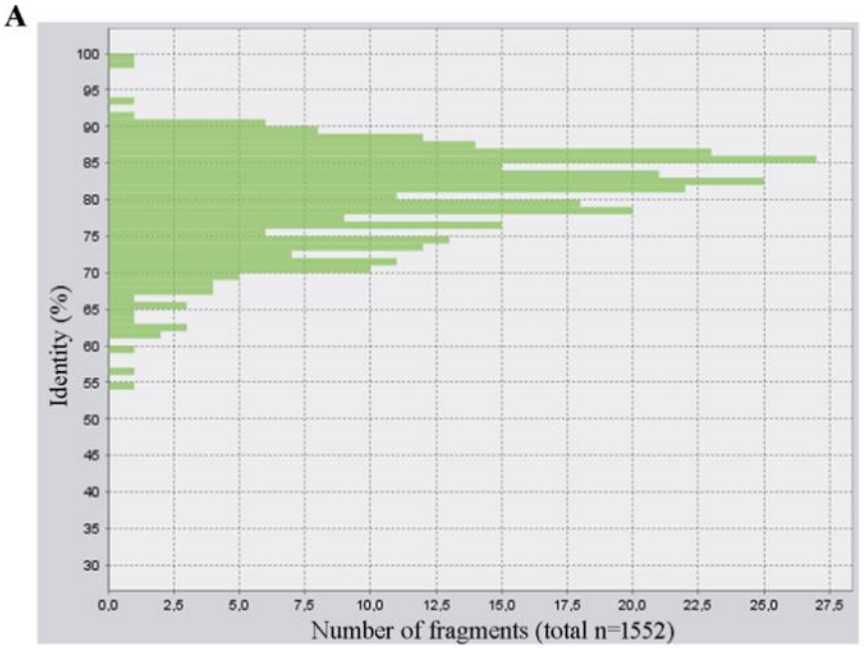
Dinucleotide frequencies PCA plot: validating our hypothesis of assignment

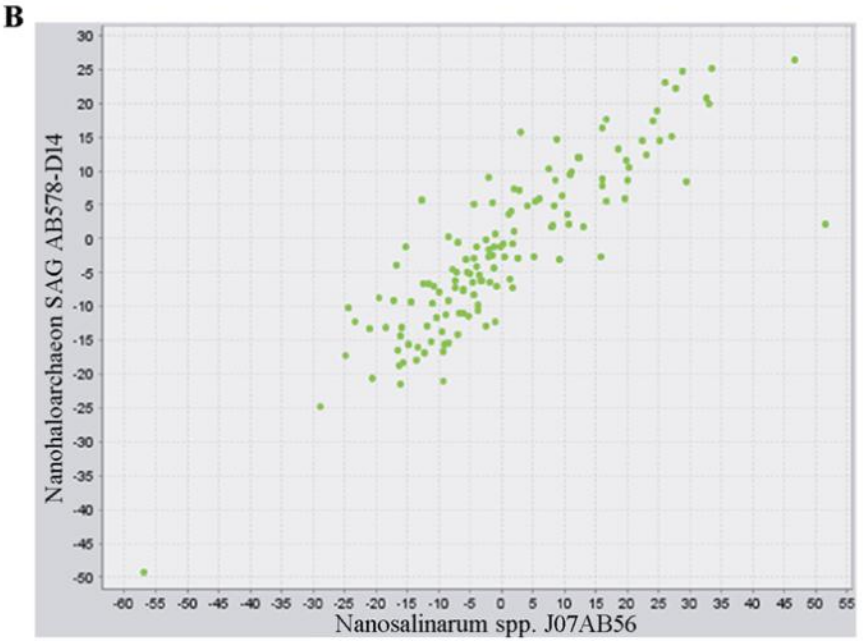


Genomic comparison: SAG AB578-D14 vs Nanosalinarum

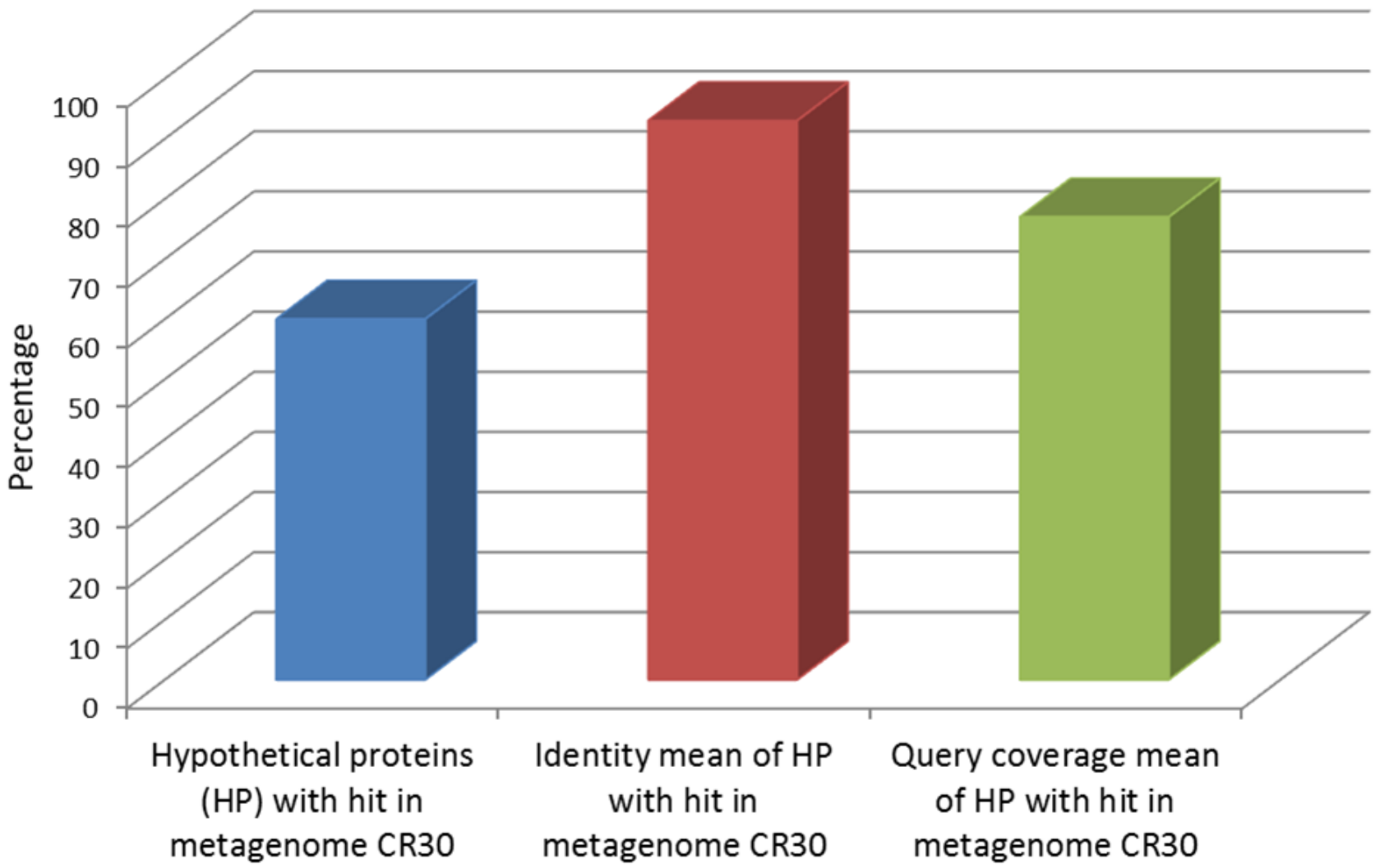


SAG AB578-D14





Most HP from SAG AB578-D14 (nanohaloarchaeon host) present in metagenome



Metagenomic data from Ghai et al., 2011

Thanks to…

Ramunas Stepanauskas and SCGC group

Pepa and her group

Bigelow Laboratory

Stefan Bertilsson

Vladimir Minim

Los Alamos National Lab

Universitat d'Alacant
Universidad de Alicante

Maine Technology Institute
Investing in Innovation